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THE THEORY OF ANÆSTHESIA.

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Anæsthesia, also termed narcosis, is a physiological condition in which the normal responsiveness or automatic activity of the living system—organism, tissue, or cell—is temporarily decreased or abolished. The subjective accompaniment of this change in higher animals is a more or less complete suppression of consciousness, with consequent insensibility to pain; the term "anæsthesia" refers more directly to this condition. By "narcosis" is usually meant a temporary paralysis or anæsthesia produced by chemical substances; this term has a more objective connotation; and is the one usually employed in purely physiological discussion. It is especially noteworthy that the condition may show all gradations of degree, ranging from a comparatively slight inhibition or insensibility to a state of profound depression in which the organism is completely inert and shows no response to even the strongest stimuli. Yet on the removal of the anæsthetizing agent the normal properties and activities return. *Reversibility* is thus an essential characteristic of the condition; this peculiarity distinguishes it from the irreversible change of death. There are, however, significant resemblances between these two states, and in fact transitions from the one to the other are frequent. Too prolonged or too profound anæsthesia may pass into death; and most anæsthetic substances, if present in too high concentration, soon cause irreversible and cytolytic changes in cells. There is in fact evidence that in many instances anæsthetic and toxic effects have the same essential physicochemical basis. The same cell-structures—especially surface-structures, *e. g.*, plasma-membranes—are primarily affected in

both cases, but in the one case the change produced is reversible, in the other irreversible. The degree of reversibility is however itself subject to variation. In many colloidal systems changes which are reversible in their earlier stages may become irreversible later; and the fact that anæsthesia, especially if profound, cannot be prolonged indefinitely without danger to life, may find its explanation here.

In any theoretical discussion of anæsthesia it is important to recognize from the first that normal or physiological conditions of reversible inhibition or suspended activity are in no sense unusual among organisms. In both animals and plants irritability and automatic activity are fluctuating properties, with a wide range of strictly physiological variation. Thus in higher animals we have conditions ranging from the profound narcosis of sleep—a state due apparently to the accumulation of fatigue-products—to one of complete mental and physical alertness or wide-awakeness. Generally speaking, responsiveness is largely a matter of metabolic condition; and most vital activities are subject to inhibition or enhancement according to the physiological requirements. Variability of this kind is in fact a necessary condition of adaptation to the changing conditions of life. Thus the activities of animals as a class are influenced to a marked degree by variations in the food-requirements. In general they become sluggish and irresponsive when well fed, and show heightened activity when deprived of food. In other words, both the automatic motor activity and the responsiveness to the stimuli of food-substances—the physiological condition expressed in consciousness as *hunger*—are increased when the supply of energy-yielding material is depleted and *vice versa*. For example, the fresh water *Hydra* shows restless swaying movements when hungry; these movements increase the area swept by the tentacles, which respond promptly to the contact of small organisms or food-particles by capturing and conveying to the mouth.[1] When well fed the creature is quiescent, and the tentacles are indifferent to such contact; they are, as it were, in an anæsthetized condition; this state passes off as the organic demand for food reasserts itself. Such an instance illustrates the regula-

[1] *Cf.* S. J. Holmes, "The Beginnings of Intelligence," *Science*, N. S., 1911, Vol. 33, p. 473.

tory rôle which fluctuations in the general responsiveness of an animal play in its normal life. Similar variations of neuro-muscular responsiveness occur throughout the animal kingdom. This is well illustrated by sleep, which is an instance of a normal or "physiological" narcosis, characterized by a definite periodicity and by affecting especially certain parts of the central nervous system; the use of opiates illustrates how readily a chemically induced narcosis may pass into the physiological form. From such facts we must conclude that the essential basis of anæsthesia consists not in a purely artificial modification of nervous or other irritability, but in some normal or physiological modification which is capable of being intensified and prolonged by the use of certain physical and chemical agencies; these are the various anæsthetizing agencies, such as the electric current, cold, or narcotizing substances. From this point of view, anæsthesia is to be regarded not as an essentially abnormal or artificial phenomenon, but simply as an intensification of a normal physiological condition; and in investigating its essential conditions we are led first to consider the normal inhibitions and depressions shown by all living cells.

Instances of such normal inhibitions are innumerable. The motor neurones innervating any group of muscles become inexcitable during the activity of the antagonist groups, as Sherrington has shown; the respiratory nerve cells cease automatic activity with over-oxygenation of the blood; vasomotor, cardiac, glandular, and muscular activities are subject to various forms of inhibition, partly nervous and partly chemical in origin. Such inhibitory mechanisms play in normal life a part whose importance is daily more widely recognized by physiologists. Mechanisms of the inverse kind, which exercise sensitizing and reinforcing influence on various functions, are also frequent in organisms. A large part of these normal inhibitions and excitations are now known to be due to chemical substances (hormones) present in the blood and derived from ductless glands or other sources of internal secretion. The regulation and integration of bodily activities are thus largely under direct chemical as well as nervous control. Such normal chemical inhibitions are probably of the same nature as artificial inhibitions due to anæsthesia.

In both cases the same kind of physico-chemical modification in the irritable element appears to form the essential determining condition.

The phenomena of anæsthesia have thus the widest biological interest; they belong chiefly in the class of chemical inhibitions or desensitizations. The inverse phenomenon of sensitization—enhancement of irritability or responsiveness—is equally widespread and plays an equally important physiological rôle. Although its study has received less attention than that of anæsthesia, its physiological interest is no less great. Irritability may in fact be altered reversibly either in the direction of increase or decrease.

It is important to note that the same substance may cause either increase or decrease of irritability or spontaneous activity, according to the conditions of concentration, temperature, physiological state of the organism, etc. In the group of lipoid-solvent substances, which include most of the anæsthetics in common use, weak solutions very generally increase excitability; stronger solutions, within a certain range of concentrations, produce typical reversible narcosis; while still stronger solutions cause cytolysis. The basis common to all of these effects requires to be determined. The problem of the general nature of anæsthesia is in fact inseparable from the wider problem of the nature and conditions of irritability in general. The essential question may be expressed thus: what is the physico-chemical basis of this property of irritability, and what conditions determine its reversible increase or decrease by chemical or other agents? This problem is one of the most fundamental in biology; and the phenomena of artificial anæsthesia are of general physiological interest largely because of the light which they throw on this larger problem.

Instances of increase in irritability or spontaneous activity under the influence of low concentrations of anæsthetic substances are frequent in both animals and plants. One of the most familiar is the general nervous excitement caused by small doses of ether, alcohol and other narcotics. Automatic rhythmical activity, as of cilia, spermatozoa, or the heart beat, is very generally heightened in weak solutions of alcohol and other

narcotics. The nerve-cells controlling the heart beat of *Limulus* show a faster rhythm in weak solutions of alcohol, chloral hydrate, choretone, and chloroform.[1] Hamburger has shown that many lipoid-soluble substances—iodoform, chloroform, turpentine, benzol, chloral hydrate, camphor, fatty acids, soaps—increase the amœboid and phagocytic activity of leucocytes, while stronger solutions decrease this activity.[2] A similar rule appears to hold for the respiratory center of vertebrates.[3] According to Vernon,[4] weak solutions of narcotics increase the consumption of oxygen in isolated tissues like the kidney. Tashiro and Adams find that low concentrations of urethane and chloral hydrate increase the excitability of nerve as well as its output of carbon-dioxide; in higher concentrations both are decreased.[5] The staircase phenomenon in irritable tissues is probably due to the stimulating action of small quantities of substances ("fatigue-substances") which in higher concentrations decrease irritability. Small quantities of alcohol increase the responsiveness of voluntary muscle and the energy of its contractions.[6] The musculature of medusæ shows increased response to mechanical stimuli in sea water containing a little alcohol.[7] Similar facts are met with in plants. Many depressant substances, when present in low concentration, increase the rate of growth.[8] Traces of ether have an accelerating or forcing influence on plant

[1] A. J. Carlson, *Amer. Journ. Physiol.*, 1906, Vol. 17, p. 182.

[2] Hamburger, "Archives Néerlandaises des Sciences Exactes et Naturelles," Serie III, B, 1911, p. 1; *Archiv für Anatomie und Physiologie*, Physiol. Abth., 1913, p. 77.

[3] *Cf.* Hamburger, "Koninklijke Akademie van Wetenschappen te Amsterdam," 1915, Vol. 17, p. 1325.

[4] *H. M. Vernon*, "The Function of Lipoids in Tissue Respiration," *Journ. of Physiol.*, 1912, Vol. 45, p. 197.

[5] Tashiro and Adams, *Internat. Zeitschr. f. physik-chem. Biol.*, 1914, Vol. 1, p. 450.

[6] *Cf.* Lee and Salant, "The Action of Alcohol on Muscle," *Amer. Journ. Physiol.*, 1902, Vol. 8, p. 61.

[7] *Cf.* Bethe, "Allgemeine Anat. u. Physiol. d. Nervensystems," Leipzig, 1903, p. 359. One half per cent. alcohol decidedly increases the mechanical irritability of the isolated central portion of the medusa *Cotalorrhiza*. F. S. Lee observed that in the Woods Hole medusa *Gonionemus* the spontaneous contractions of the swimming bell are markedly increased by small quantities of alcohol (1/16 to 1/4 per cent.); *cf. Amer. Journ. Physiol.*, 1903, Vol. 8, p. xix.

[8] Numerous instances of this effect are cited by Czapek, *Biochemie der Pflanzen*, Jena, 1913, p. 148.

growth,—a fact of which practical use is made by horticulturists. Increase in oxygen-consumption under the influence of chloroform and ether has been observed by Elfving and others; higher concentrations decrease oxygen-consumption.[1] Demoor and others have observed an acceleration of protoplasmic rotation in plant cells during the early stages of chloroform and ether narcosis; alcohol also causes this effect.[2] Traces of ether increase the irritability of sensitive plants (*Mimosa*);[3] higher concentrations cause typical anæsthesia.[4]

A probably related phenomenon is seen in certain artificial modifications of response induced in various organisms by weak solutions of anæsthetics. A striking instance is the reaction of many lower animals to light. Loeb has found that *Daphniæ*, which normally show little or no directive light-response, become positively heliotropic in weak solutions of alcohol and other narcotics, in concentrations of a third to a half of those required for anæsthesia.[5] Similarly I have found that the larvæ of the marine annelid *Arenicola*, which normally exhibit strong positive heliotropism, become *negative* in weak solutions of various anæsthetic substances. Similar observations have been made by Torrey, A. R. Moore, and other observers.

The phenomenon of reversible *decrease* of activity or responsiveness is anæsthesia. The vital processes subject to such reversible arrest are of the most varied kind. They include

[1] *Cf.* Czapek, *loc. cit.*, p. 159, for instances of this effect. Tashiro and Adams (*loc. cit.*) cite observations of Kosinski showing that respiration in yeast cells is increased in presence of 0.5 per cent. ether; 5 per cent. reduces respiration one half, while 7 per cent. almost stops it. Baer and Meyerstein find increased oxidation of oxy-butyric acid to acetone in the perfused liver under the influence of various compounds which in higher concentrations check oxidations, *e. g.*, tricholor-alcohol, *p*- and *m*-oxy-benzoic acid, *p*-oxy-benzaldehyde (*cf.* p. 458 of their paper in *Arch. exper. Path. u. Pharm.*, 1910, Vol. 63).

[2] *Cf.* the instances cited by Czapek, p. 161. H. Nothmann Zuckerkandl has also observed this effect with low concentrations of alcohol and ether (*cf.* footnote 2, p. 317).

[3] Personal communication from Professor J. M. Macfarlane, of the University of Pennsylvania.

[4] *Cf.* Claude Bernard, "Lecons sur les phénomènes de la vie communs aux animaux et végétaux," Paris, 1878. Anæsthesia of plant-growth was also studied by Bernard.

[5] J. Loeb, *Biochem. Zeitschr.*, 1909, Vol. 23, p. 93.

amœboid movement;[1] protoplasmic rotation in plant cells;[2] all processes depending on response to stimulation, like muscular contraction and stimulation and conduction in nerve; automatic rhythmical activities like the heart beat or the motion of cilia or spermatozoa; cell-division;[3] the artificial initiation of development in unfertilized eggs;[4] the stimulating, cytolytic or other physiological action of salt solutions;[5] various fermentative and oxidative processes;[6] light-production, *e. g.*, by luminous bacteria;[7] typical metabolic processes like the assimilation of carbon dioxide by plants;[8] growth processes in plants and animals, and developmental processes dependent on growth and cell-division. It is especially worthy of note that not only motor activity and responsiveness are subject to control of this kind, but also processes like growth and development. The growth of seedlings may be temporarily arrested by ether in sufficient concentration, as Claude Bernard showed.[9] Cell-division in the eggs of sea-urchins is checked by anæsthetics in concentrations of the same order as those required for neuro-muscular anæsthesia in Arenicola larvæ.[10] It is thus not surprising that developmental

[1] *Cf.* Hamburger: *loc. cit.*

[2] *Cf.* H. Nothmann-Zuckerkandl: *Biochem. Zeitschr.*, 1912, Vol. 45, p. 412.

[3] *Cf.* (*e. g.*) my observations on anæsthesia of cleavage in sea-urchin eggs, *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121. The development of astral radiations in dividing egg-cells is prevented by etherization, and existing radiations are suppressed: *cf.* E. B. Wilson: Arch. f. Entwicklungsmechanik, 1901, Vol. 13, p. 353.

[4] R. S. Lillie, *Journ. Exper. Zool.*, 1914, Vol. 16, p. 591.

[5] *Cf.* my papers on antagonisms between salts and anæsthetics; *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 372; Vol. 30, p. 1; 1913, Vol. 31, p. 255.

[6] *Cf.* the papers of Warburg: *Zeitschrift f. physiol. Chemie*, 1910, Vol. 69, p. 452, and Vol. 70, 1911, p. 413; *Pflüger's Arch.*, 1914, Vol. 155, p. 547; Warburg and Wiesel, 1912, Vol. 144, p. 472; Usui, *ibid.*, 1912, Vol. 147, p. 100; Meyerhof, *Pflüger's Archiv*, 1914, Vol. 157, p. 251. Claude Bernard describes the reversible inhibition of yeast-fermentation by anæsthetics (*cf.* footnote 9, below).

[7] E. N. Harvey, BIOLOGICAL BULLETIN, 1915, Vol. 29, p. 308.

[8] *Cf.* Claude Bernard, *loc. cit.*; Overton, "Studien über die Narkose," p. 182.

[9] *Loc. cit.* In Bernard's address, "La Sensibilité," given in 1876 before the French Association for the Advancement of Science, and published in his book, "La Science Expérimentale," Paris, 1890, he cites instances of anæsthesia of the most various vital processes, including photosynthesis, germination and growth in plants, fermentation by yeast, development of the hen's egg,—and concludes: "We may say that everything living is sensitive and can be anæsthetized; whatever is not sensitive is not living and cannot be anæsthetized "(p. 224).

[10] R. S. Lillie, *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121.

processes, depending as they do on cell-division and growth, are similarly subject to inhibition by anæsthetics. Stockard and McClendon[1] have shown that such substances induce abnormalities like cyclopia in developing fish eggs, an effect which is to be referred to the arrested development of certain portions of the central nervous system, especially the anterior region of the fore-brain between the optic vesicles. Abnormalities of growth and development as well as of irritability may thus be produced under the influence of anæsthetics. Since an automatic power of growth—*i. e.*, increase in specifically organized and metabolically active material—is perhaps the most fundamental manifestation of vital activity, the fact that it is subject to reversible arrest by anæsthetic substances is of the greatest biological significance, and illustrates in a striking manner the unity of the conditions which control the most various cell-processes. We may infer that in the general course of constructive as well as of destructive metabolism, processes are concerned which are identical with those underlying the ordinary manifestations of stimulation. These latter, however, are almost certainly dependent on surface-changes, of which the most essential are probably variations in the electrical polarization of the plasma-membranes (see below, p. 365). The controlling influence of membrane-processes in such fundamental physiological activities as growth and assimilation is thus indicated by this susceptibility to arrest by anæsthetics.

In any complete theoretical discussion of anæsthesia it is necessary first to consider the chief conditions under which living cells in general undergo reversible decrease or loss of irritability. This change occurs under a variety of external conditions, mechanical, thermal, electrical and chemical. Me-

[1] *Cf.* C. R. Stockard, *Archiv f. Entwicklungsmechanik*, 1907, Vol. 23, p. 249; *Anatomical Record*, 1909, Vol. 3, p. 167 ("The Artificial Production of One-eyed Monsters . . . by the Use of Chemicals"); *Amer. Journ. Anat.*, 1910, Vol. 10, p. 369 ("The Influence of Alcohol and other Anæsthetics on Embryonic Development"). Also McClendon ("Physical Chemistry of the Production of One-eyed Monstrosities") in *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 289. Stockard observed the production of these abnormalities first with magnesium salts, later with lipoid-solvent anæsthetics (alcohol, ether, chloroform, chloretone). Developmental defects are also produced in mammals by alcohol (*cf.* Stockard, *Arch. f. Entwicklungsmech.*, 1912, Vol. 35, p. 569; *Amer. Naturalist*, 1913, Vol. 47, p. 641).

chanical shock may cause temporary loss of irritability. This is probably an effect of over-stimulation and due to prolongation of the refractory period; it resembles in some respects the effect produced in voluntary muscle by poisons like veratrin, which greatly prolongs the relaxation phase and the recovery of irritability following contraction. The paralysis due to mechanical shock differs however from that of anæsthesia in important respects; it represents an injury from which the cell can recover, while true unmixed anæsthesia is quite without injurious action. Certain effects of altered temperature have a closer resemblance to anæsthesia. Most cells and tissues, within the range of temperature in which they show normal activity, show decreased automatic activity with decrease of temperature. Thus according to Snyder[1] the heart of the tortoise shows eighteen beats per minute at 20° and thirty-five at 30°. Observations on the hearts of other animals have given similar results.[2] Within the physiological range of temperature the rate is doubled or trebled by a rise of 10°. This rate of change of velocity with temperature, or temperature-coefficient, is characteristic of chemical reactions in general and is not a distinctively physiological phenomenon. Metabolic and hence vital activity is slowed by cooling just as any other chemical process is slowed. The same temperature-coefficient is shown by a large number of physiological processes including cell-division, rate of conduction in nerve, enzyme action and many others.[3] Thus the above effect of cold is dependent simply on a slowing of chemical processes in cells and has in it nothing distinctively vital. It is important, however, to consider this effect in relation to the problem of anæsthesia, for a simple decrease in reaction-velocity, due to the presence of anti-catalytic substances, is held by various investigators to be the essential condition of anæsthesia. Decrease in the rate of a physiological process, like the heart beat, or muscular contraction, or the spread of the excitation-wave in nerve, is not how-

[1] University of California Publications, Physiology, 1905, Vol. 2, p. 125.

[2] *Cf.* C. D. Snyder, *Amer. Journ. Physiol.*, 1906, Vol. 17, p. 350; *Zeitschr. f. allg. Physiol.*, 1912, Vol. 14, p. 263; Robertson, BIOL. BULL., 1906, Vol. 10, p. 242; C. G. Rogers, *Amer. Journ. Physiol.*, 1911, Vol. 28, p. 81; BIOL. BULL., 1914, Vol. 27, p. 269; Loeb and Ewald, *Biochem. Zeitschr.*, 1910, Vol. 28, p. 340.

[3] For instances *cf.* Snyder, "Temperature-coefficients of Various Physiological Actions," *Amer. Journ. Physiol.*, 1908, Vol. 22, p. 309.

ever necessarily associated with a change in the irritability and other vital properties of the tissue; in fact moderate cooling may increase the irritability of nerve. Irritability and rate of metabolic processes represent in fact two independent variables. We infer that anæsthesia is not simply an expression of a decrease in the velocity of certain chemical reactions, such as oxidations, but that some other factor enters, probably physical in nature. Certain other effects of temperature bear a closer resemblance to true anæsthesia. Various irritable tissues become reversibly insensitive at temperatures slightly below or above the normal physiological range. Thus the frog's heart shows an accelerated rate with rise of temperature up to 36° or 37°; it then becomes temporarily inactive and insensitive ("heat-standstill"), but resumes beating if the temperature is lowered. Similarly the musculature of tropical medusæ becomes irresponsive at 40° and recovers on lowering the temperature.[1] This condition of reversible heat-paralysis has certain suggestive resemblances to anæsthesia. Cooling may produce a similar loss of sensitivity in cells whose normal temperature is high, as those of tropical marine animals[2] or warm-blooded vertebrates. Sensory nerve endings, musculature, etc., lose sensitivity if cooled sufficiently, and recover on warming. In these effects structural alterations due to modification of the colloids of the cells (as gelation) are probably concerned; and, as will be shown later, there are indications that similar changes form part of the essential basis of true anæsthesia. The fact that changes of temperature may thus alter the irritability of the tissue independently of their influence on reaction-velocity as such, is highly important to the general theory of narcosis; and it appears unfavorable to those theories which refer anæsthesia to a simple change in the rate of chemical processes like oxidation. Recent experiments by Loeb and Wasteneys[3] on sea-urchin eggs illustrate this. They found that during a condition of narcosis sufficient to arrest cell-division completely, the rate of oxidation is lowered by only 10 per cent.;

[1] *Cf.* E. N. Harvey, Carnegie Institution Publications, No. 132, 1910, p. 32.

[2] *Cf.* A. G. Mayer, "Effects of Temperature upon Tropical Marine Animals," Carnegie Institution Publications, No. 183, 1914, p. 1.

[3] *Journ. Biol. Chem.*, 1913, Vol. 14, p. 517; *Biochem. Zeitschr.*, 1913, Vol. 56, p. 295.

the same effect on the rate of oxidation results from a simple lowering of temperature by 2° to 3°, a change which only slightly retards cell-division. Decrease in the rate of oxidation as such is thus quite insufficient to account for the inhibitory effect. The fact that, *e. g.*, in frogs' muscle a lowering of temperature of 20° (*e. g.*, from 35° to 15°)—which reduces the rate of oxidation to one fifth of its former value—leaves irritability unimpaired, indicates that any explanation of anæsthesia based on simple decrease in reaction-velocity is inadmissible. A similar decrease in the rate of oxidation can be produced by lipoid-solvent anæsthetics only in concentrations which are much higher than those requisite for anæsthesia.

The constant electric current produces in many irritable tissues effects closely resembling true anæsthesia. Many physiological inhibitions may be caused by passing a constant current through the tissue. There is indeed reason to believe that many of the normal inhibitions, *e. g.*, in the neurones of reflex arcs, are electrical in their nature.[1] The anti-stimulating or desensitizing action of the constant current thus deserves careful consideration in any general theory of anæsthesia. As is well known, the action of the current on irritable tissues like nerve and muscle is *polar*; where the current enters the tissue there is decreased irritability, depression, or inhibition (anelectrotonus); where it leaves there is excitation or heightened irritability (catelectrotonus). Thus a nerve becomes inexcitable near the anode when the constant current is passed; under similar conditions the heart is inhibited and voluntary muscle relaxed. The condition is reversible, and in fact constitutes a typical local anæsthesia. The essential basis of the effect appears to be an altered electrical polarization of the cell-surface. Near the anode, where the current enters the cell or irritable element, the normal outer positivity of the semi-permeable plasma-membrane is increased. Apparently this change renders the membrane irresponsive to stimulation. Variations in the electrical polarization of the plasma-membrane are in all probability constantly associated with variations in irritability. The facts of electrotonus show that such changes of polarization may profoundly alter the

[1] *Cf.* my discussion of this possibility in *Amer. Journ. Physiol.*, 1913, Vol. 31, pp. 284 *seq.*

irritability and automatic activities of the cell. This general conception is of the greatest importance in the theory of anæsthesia, and will be reconsidered later.

The most important instances of anæsthesia are those produced by chemical substances. First it should be noted that substances belonging to the most various classes may have anæsthetic effects. This fact is overlooked in theories like those of Overton and Meyer, Traube, and others, which refer anæsthesia to the special properties of lipoid-solvent substances, which are regarded as acting either by dissolving in the lipoid constituents of the cell or by adsorption at the surfaces of membranes or other structures. The anæsthetic influence of certain neutral salts shows, however, that lipoid-solubility or surface activity is not essential to narcotic action; magnesium sulphate has long been used by naturalists to narcotize marine animals; more recently it has been applied by Meltzer to produce spinal anæsthesia in mammals. Similar reversible depressant effects are produced by potassium salts. Salts of calcium and strontium also cause reversible desensitization of isolated nerve and muscle. In most animals the calcium-content of the medium has marked influence on irritability and automatic activity; this is well shown in the case of vertebrate muscle; lowering the ratio of calcium to sodium in indifferent media like Ringer's solution has a sensitizing effect, and if the calcium falls too low the muscle twitches spontaneously; increasing the calcium-sodium ratio has a desensitizing action; these effects are reversible.[1] Calcium also antagonizes the stimulating and sensitizing action of pure solutions of sodium and other salts on muscle and nerve. Similarly the heart beats best in media of a certain calcium-content. In marine medusæ (*Rhizostoma* according to Bethe) the rhythmical beat ceases when the animal is transferred to calcium-free sea water, and is restored if calcium is added; still further addition of calcium again arrests the movement.[2] These facts make it clear that alteration of the salt-content of the media may have effects essentially identical with anæsthesia. This is a fact of much theoretical interest, since it indicates that the general condition of the colloids of the cell,

[1] For instances of these various effects *cf.* J. Loeb's article on "Physiological Actions of Ions," in Oppenheimer's "Handbuch der Biochemie," 1909, Vol. 2, p. 104.

[2] *Cf.* Bethe, *Pflüger's Archiv*, 1909, Vol. 124, p. 561.

especially of the surface-layer or plasma-membrane, is a chief factor in determining the irritability and automatic activity of the living cell. Further evidence of this will be given later. Modification of the properties of this layer may result from an alteration in the state of either its lipoid or its protein constituents, and if this alteration is reversible a temporary inhibition, or anæsthesia, may result. A related condition is seen in the irritable tissues of higher animals, such as muscle and nerve. In these tissues irritability depends on the presence of certain salts in the media; simple withdrawal of salts and replacement by indifferent non-electrolytes like sugar is followed by a temporary loss of irritability; the latter is restored by return to media containing salts, especially sodium salts.[1] The musculature of marine animals (*e. g.*, *Arenicola* larvæ) is similarly inactivated in isotonic solutions of non-electrolytes, and regains irritability in isotonic solutions of various neutral salts. Solutions of sodium salts, together with a small proportion of calcium, are especially favorable. Sodium may be partly replaced by lithium, but not by other metals.[2] Thus the presence of certain salts in the medium is necessary for normal irritability,—hence the effects of isotonic sugar solution, which are due to the absence of salts, not to any special action of the non-electrolyte. The salt-content of the medium may be reduced to a small fraction—one tenth or less—of the normal by diluting the physiological salt solution with isotonic sugar solution, without causing loss of irritability. But with the complete withdrawal of salts irritability soon disappears. In cases like this, where normal irritability is *dependent* on the salt-content of the media, modification of the latter may induce a reversible desensitization closely resembling anæsthesia. Probably several factors enter in the production of this effect, of which the two chief are, a direct change in the properties of the plasma-membrane (colloidal consistency, electrical polarization), and a lowering of the electrical conductivity of the medium.

The reaction of the medium (H-ion concentration) also has profound influence on the irritability and automatic activity of many cells; and a reversible suspension of function akin to

[1] *Cf.* Overton, *Pflüger's Archiv*, 1902, Vol. 92, p. 346, and 1904, Vol. 105, p. 176.

[2] R. S. Lillie, *Amer. Journ. Physiol.*, 1909, Vol. 24, p. 459.

anæsthesia may result from a slight change in this reaction. In higher vertebrates the normal reaction of the blood plasma is not far from neutral, and varies only slightly from a constant normal value ($C_H = 0.35 \times 10^{-7}$ to 0.5×10^{-7}); but certain cells of the central nervous system are especially sensitive to such variations. The activity of the respiratory center is apparently regulated by the variations in the H-ion concentration of the blood, cessation of activity resulting from a slight decrease (*i. e.*, increased alkalinity), and increased activity from a slight increase.[1] Reversible cessation of activity may thus result from a slight change in reaction, due, *e. g.*, to loss of CO_2. Similar conditions are known to exist in certain marine animals; thus according to Bethe,[2] slight increase in the alkalinity of the sea water arrests, while slight acidulation accelerates, the rhythmical contraction of medusæ. On the other hand, the activity of many cells and tissues is favored by slight increase in external alkalinity, and depressed by slight acidulation. The irritability and automaticity of living cells are thus largely a function of the reaction of the medium, and this fact has an intimate bearing on the question of the mechanism of anæsthetic and other inhibitions. The precise physico-chemical basis of this action is uncertain, but it probably depends chiefly on alterations in the electrical polarization of the cell-surface. Slight variations in alkalinity or acidity are known to produce marked effects on the electrical polarization of surfaces bathed by media of approximately neutral reaction.[3]

The chief chemical substances exerting a reversible depressant influence on a wide range of vital activities are those numerous and chemically diverse organic compounds of which the most evident common property is a solvent action on, or solubility in, fats and fat-solvents. Substances of this class form the majority of anæsthetics in common use; they include alcohols, ethers, esters, aldehydes, ketones, nitriles, amides, various normal and substituted hydrocarbons (chloroform, benzol, etc.) and other related compounds. Most of these bodies are members of

[1] For a recent review and discussion of the evidence *cf.* Winterstein, *Biochem. Zeitschr.*, 1915, Vol. 70, p. 45.

[2] *Pflüger's Archiv*, 1909, Vol. 127, p. 219.

[3] *Cf.* Haber and Klemensiewicz, *Zeitschr. f. physik. Chemie*, 1909, Vol. 67, p. 385.

homologous series; and it is highly characteristic of such series that the ratio of oil-solubility to water-solubility (oil-water partition-coefficient) increases regularly with increase in molecular weight. At the same time the narcotizing power increases; *i. e.*, in any single series (*e. g.*, alcohols) the higher the molecular weight the lower the concentration required for narcosis. It was this general parallelism that led Overton and Meyer to the view that anæsthetic power, in the case of any substance, is a direct function of its solubility in the fat-like or lipoid constituents of the cell. That a connection exists between the fat-solvent and the anæsthetic properties of a compound had previously been suggested by Bibra and Harless in 1847, and the same view was later expressed by Hermann, C. Bernard, Richet, Ehrlich and others.[1] The first systematic studies of this relationship were however those of Overton and Meyer, the results of whose experiments, carried on independently, were published about the same time (1899).

In a study of the permeability of animal and plant cells to various types of compounds, Overton[2] had reached the conclusion that solubility in lipoids was the chief factor determining the ready entrance of a compound into cells; compounds with well-marked power of penetration belonged chiefly to the narcotic group; and in a later extensive investigation on narcosis in tadpoles[3] a far-reaching parallelism was found between the oil-water partition-coefficients of a wide range of organic compounds and their narcotizing action. The nature of Overton's results may be best seen from the following series, which gives the concentrations required to narcotize tadpoles in the case of the ethyl esters of the first five fatty acids. (See Table I.)

The narcotic action is seen to increase steadily with decrease in the water-solubility,—*i. e.*, increase in the ratio of partition between oil and water. Each member of the series is from two to three times as effective as its immediate predecessor. This rule appears to hold very generally for members of homologous

[1] For an account of these earlier views *cf.* Overton, "Studien über die Narkose," June, 1901.

[2] Overton, "On the General Osmotic Properties of Cells," etc., *Vierteljahrsschr. d. naturf. Gesellsch. in Zürich*, 1899, Vol. 44, p. 88.

[3] "Studien über die Narkose," 1901.

series, and a large number of similar instances have been collected by Traube and other recent investigators.[1] Numerous other experiments with alcohols, hydrocarbons, aldehydes, ketones, etc., showed a similar increase in narcotic action with increase in the oil-water partition-coefficients. Overton accordingly drew the conclusion that narcotics act by *dissolving* in certain substances, contained especially in nerve-cells, which resemble fats in their solvent properties; these substances are the lipoids, especially lecithin and cholesterin, which appear to be essential constituents of protoplasm; it is the *physical* modification of these substances, due to their being charged or impregnated with the lipoid-soluble narcotic, that forms the essential condition of anæsthesia. Meyer's conclusion was similar;[2] the narcotizability of cells is thus related to the nature and the proportion of the lipoids present in the protoplasm; the high susceptibility of nerve-cells is probably dependent on their high lipoid-content. The unequal action of different narcotics depends on their unequal partition-coefficients, which determine their distribution in a mixture of water and lipoid substances. The greater the relative lipoid-solubility the larger the proportion of the anæsthetic present in solution in the lipoid cell-constituents when the partition-equilibrium is reached. Hence, if the lipoid-solubility of a substance is very high, extremely dilute solutions may exert anæsthetic action. Overton, for example, found that phenanthrene could narcotize tadpoles in dilutions so low as one part in 1,500,000 of water.

TABLE I.

Ester.	Narcotizing Concentration. (Mols per Liter).	Solubility in Oil and Water.
Ethyl formate	0.07*m*–.09*m*	Oil: water = 4 : 1
" acetate	.03*m*	In 15.2 parts water; in all parts oil
" propionate	.01*m*–.012*m*	" 50 " " " " " "
" butyrate	.0043*m*	" 190 " " " " " "
(" isobutyrate)	.0057*m*	" 140 " " " " " "
" valerianate	.0019*m*	" 500 " " " " " "

Overton's study of permeability had led him to the conclusion that the outer layer or plasma-membrane of cells consists largely of lipoid material; in this way he explained the ready entrance

[1] *Cf.* Traube, "Theorie der Narkose," *Pflüger's Archiv*, 1913, Vol. 153, p. 276.

[2] Hans Meyer, *Arch. f. exper. Path. u. Pharm.*, 1899, Vol. 42, p. 109.

of lipoid-soluble substances into cells. Now it is an evident corollary of Overton's hypothesis that if the anæsthetic acts by changing the physical state of the lipoid cell-constituents it must affect the properties of a lipoid-rich cell-structure like the plasma-membrane. Overton, however, does not refer narcotic action specifically to a modification of the plasma-membrane alone, but to a general modification in the physical state of all cell-lipoids, wherever situated. Recently, however, much evidence has accumulated indicating that the essential influence is that exerted on the plasma-membrane, and that it is the modification in the properties of this structure which determines the characteristic anæsthetic effect. This evidence and its implications will be considered later.

The hypothesis of Overton and Meyer has received wide acceptance. It is not clear, however, why simple solution of chemically indifferent substances in the lipoids of the tissue should so modify its irritability; and Overton and Meyer do not attempt to explain this connection. The parallelism between lipoid-solubility and narcotic action is not an exact one, and many exceptions to the rule are known. The powerful narcotic action of chloral hydrate, which is several times more soluble in water than in oil, is not thus explained; and lipoid-insoluble neutral salts of magnesium and other metals may exert typical narcotic action. Evidently other factors than solubility may enter. Yet the evidence adduced by Overton and Meyer, as well as by more recent investigators, leaves no doubt that in the case of organic anæsthetics high lipoid-solubility is typically associated with marked narcotic action. The reversibility of anæsthesia corresponds to the reversibility of the process of solution. The chemical indifference of many anæsthetics is thus not surprising, since the substance acts not by chemical combination but by simple solution in the cell-lipoids.

According to Overton and Meyer's hypothesis it is this *solution* of the narcotic in the lipoid which determines anæsthetic action. This view has recently been attacked from various sides. According to Traube,[1] the anæsthetic acts not by *dissolving* in the

[1] I. Traube, "Theorie der Narkose," *Pflüger's Archiv*, 1913, Vol. 153, p. 276, and Vol. 160, 1915, p. 501; also "Theorie des Haftdrucks und Lipoidtheorie," *Biochem. Zeitschr.*, 1913, Vol. 54, p. 305, and other papers cited there.

cell lipoids, but rather by undergoing surface-condensation or adsorption at the physiologically active surfaces within the living system; these may be the surfaces of special cell-structures or of colloidal particles, whether lipoid or protein. The catalytic activity of these surfaces is thus decreased, and the reaction-velocities of essential chemical processes, especially oxidations, is lowered. A corresponding depression of cell-functions results. Whether this effect is to be attributed to a displacement of metabolically active water-soluble substances like sugar, whose surface-activity is relatively small, or to a direct alteration in the catalytic properties of the physiologically active surfaces themselves, is uncertain. The essential feature of Traube's view is that it regards the *surface-activity* of a narcotic compound, *i. e.*, its influence in lowering surface-tension—rather than its lipoid solubility—as the determining factor in its depressant action. This surface-activity determines the degree of adsorption, and hence, indirectly, of anæsthetic action. It is well known that the surface-tension of such a solvent as water, in contact with air or with another liquid or a solid, is greatly influenced by the presence of dissolved substances. This influence is usually in the direction of a decrease. A few substances like inorganic salts and sugars increase the surface-tension of water, although the effect is slight; but the majority, especially of organic substances, cause well-marked and often great decrease. This is especially true of substances whose water-solubility is limited; and in general the more soluble a substance is in oils or other water-insoluble organic solvents, and the less soluble in water, the greater is its influence (for a given molecular concentration) on the surface-tension of water. In a capillary tube the level of pure water or of an aqueous solution is raised, by the contractile force or tension of the surface of the water-film lining the walls of the tube, to a certain height above the level of the water outside. This height (h) is proportional to the surface tension of the water (σ), and inversely proportional to the radius of the tube (r) and the specific gravity (g) of the liquid ($h = 2\sigma/rg$). The relative surface-tensions of aqueous solutions may thus be determined by measuring the heights to which the column of solution is raised by capillarity in a given tube. This

height is decreased by surface-active substances, and the degree of capillary activity or tension-lowering action of different substances can thus be determined. Now in any homologous series this action (for equimolecular solutions) is found to decrease as the molecular weight increases. The surface-tension in milligrams per linear centimeter (*i. e.*, the pull exerted by a strip of surface one centimeter wide) of $m/4$ solutions for the first five aliphatic alcohols at 15° is given by Traube as follows: the surface-tension of the $m/4$ solution of dextrose, a physiologically important surface-inactive compound, is given for comparison.

TABLE II.

Liquid (Solutions = m/4).	Surface-tension (Milligrams per Centimeter).	Molecular Concentrations of Isocapillary Solutions.	Concentration for Narcosis of Tadpoles (Overton).
Water	73		
$m/4$ dextrose	73.3		
Methyl alcohol	70.5	14.0	$0.52m$–$0.62m$
Ethyl "	67.3	5.0	$0.27m$–$0.31m$
n-propyl "	58.9	1.6	$0.11m$
i-butyl "	44.9	0.46	$0.045m$
i-amyl "	30.5	0.14	$0.023m$

The second column gives the concentrations required to effect a definite lowering of surface-tension. It will be observed that the surface-activity of each member (as measured by the reciprocals of the isocapillary concentrations) is approximately a third of that of its immediate successor. The third column shows that the narcotic activity increases from each member to the next following in a closely similar proportion. Results of this kind are on the whole typical for homologous series. The question arises as to their general physiological significance.

According to Traube the essential physico-chemical factor in these physiological effects is the characteristic influence which surface-tension has upon the distribution of dissolved substances in any polyphasic system. The general principle of Willard Gibbs and J. J. Thomson states that substances which lower the surface-tension of any solvent attain, when equilibrium is reached, a higher concentration in the surface-layer than in the interior of the solvent; a surface-condensation or adsorption thus results, which is the greater the greater the surface-activity of the dis-

solved compound. Hence substances having a high degree of surface-activity are as a class readily adsorbed. The effect is the same as if a relatively slight coherence existed between the solvent and the dissolved substance. Hence Traube conceives of a surface-active substance as one in which the union or adhesion between solute and solvent is slight; *i. e.*, relatively little work is required to separate the substance from solution; and he has introduced the expression "Haftdruck" (adhesion-tension or solution-affinity) to designate this condition. The capillary activity of any substance in a given solvent varies inversely with its solution-affinity (Haftdruck) relatively to that solvent. The lower the solution-affinity relatively to water the greater is the tendency of any substance to pass out of its solution in water; this tendency favors the entrance of capillary-active substances into other adjoining solvents or media, *e. g.*, into and through the membranes bounding cells.[1] The ready penetration of such substances into living cells is in fact referred by Traube not to lipoid-solubility, but to low solution-affinity in relation to the medium bathing the cell. A tendency to surface-condensation or adsorption is a characteristic accompaniment of low solution-affinity to water; the marked physiological activity shown by surface-active substances as a class is a direct consequence of this tendency.

According to data already cited, the narcotic activity of organic substances shows a parallelism with both capillary activity

[1] Traube's attempts to apply this conception to a general explanation of osmotic phenomena are not convincing. The fact that lipoid-solubility varies in the same direction as capillary activity makes the question difficult to decide by experiments on living cells. But in the case of dead cells, as well as of more permeable partitions like parchment paper or collodion, surface-active and surface-inactive substances appear to penetrate with equal ease. The difference between the rate of penetration of dissolved lipoid-insoluble substances into living and into dead cells shows that the permeability of the partition (*i. e.*, relative resistance to diffusion) is the deciding factor in their entrance. Furthermore, instances are well known where the rate of penetration of a substance through an artificial partition varies directly with its solubility in the material composing the partition. Flusin's experiments with rubber membranes are a good instance of this. The diffusion of substances through the surface-films bounding the cells implies a passage either through or between the membrane-constituents; and in the case of the living cell, solution of lipoid-soluble substances in the lipoids of the membrane is probably the main factor in their entrance, although this entrance may be favored by adsorption due to surface-activity.

and lipoid-solubility. Other physiological effects (*e. g.*, membrane-formation in sea-urchin eggs,[1] reversal of the sense of heliotropism, sensitizing action, cytolytic action) show a similar parallelism. The question of whether the particular physiological effect under consideration is determined by one or the other factor, or by the interaction of both, has to be decided by further evidence. In favor of the view that lipoid-solubility rather than surface-activity is the essential determining factor in the action of lipoid-soluble narcotics, is the fact that the action varies with temperature in the same direction as the oil-water partition-coefficient. This is shown in a remarkable manner in the following table from Hans Meyer.[2] The concentrations required to narcotize tadpoles were determined at the two temperatures 3° and 30° using (*a*) narcotics whose relative solubility in oil *decreases* with rise in temperature, and (*b*) where it *increases*. The critical anæsthetizing concentrations for the following six anæsthetics are given in the table.

TABLE III.

Anæsthetic.	Critical Concentration for Anæsthesia.		Oil/Water Partition-Coefficients.	
	At 3°.	At 30°.	At 3°.	At 30°.
A. Salicylamide.....	*m*/1300	*m*/600	22.23	14
Benzamide.....	*m*/500	*m*/200	0.67	0.43
Monoacetin.....	*m*/90	*m*/70	0.099	0.066
B. Ethyl alcohol.....	*m*/3	*m*/7	0.026	0.047
Chloral hydrate.....	*m*/50	*m*/250	0.053	0.236
Acetone.....	*m*/3	*m*/7	0.146	0.235

In the first three compounds the relative lipoid-solubility decreases and the narcotizing concentration increases with rise of temperature; while with the others the conditions are reversed. Thus simple cooling suffices to restore activity to tadpoles anæsthetized in chloral hydrate at 30°. If adsorption under the influence of surface-tension were the main factor in these effects, such a change of narcotic power with temperature would be inexplicable, since surface-tension is influenced in a totally different manner by change of temperature. The fact that

[1] *Cf.* J. Loeb, "Artificial Parthenogenesis and Fertilization," University of Chicago Press, 1913, Chapter 14.

[2] H. Meyer, *Arch. f. exper. Path. u. Pharm.*, 1901, Vol. 46, p. 338.

anæsthetics collect in cells in higher concentration than in the medium also favors the partition rather than the adsorption theory of narcosis. Chloroform, ether, and esters undergo concentration in nervous and other tissues, as Pohl[1] and Hedin[2] have shown. Warburg and Wiesel[3] have also found in the case of yeast that the tendency of compounds to concentrate in cells increases with increase in their narcotic power. In solutions that diminished fermentative activity by one half, phenyl urethane was found to be three times and thymol nine times more concentrated in the cells than in the medium. These facts strongly suggest a distribution according to relative solubilities.

What Traube especially insists upon is that effects similar to narcosis are shown in cases where lipoid-solubility can play no part. Thus according to Warburg and Wiesel[4] the fermentative and oxidative activities shown by lipoid-free preparations of dried microörganisms are influenced by the lipoid-solvent anæsthetics in the same manner as in the intact organisms. It is to be noted, however, that the effective concentrations are much higher in the case of such preparations than in that of living cells. Traube cites a large number of observations made with solutions of various surface-active substances, showing that with both animal and plant cells, as well as with enzymes, the degree of narcotic and cytolytic action—of inhibition and destruction in the case of enzymes—is nearly proportional to the surface-activity of the solution.[5] Solutions of widely different substances, provided they have the same surface-tension ("isocapillary" solutions), have equal physiological action. In the following table I have collected a number of observations illustrating the various physiological effects produced by members of the aliphatic alcohol series. In each instance the molecular concentrations required to produce a definite physiological effect are given; the molecular concentrations which cause equal lowering of surface-tension (isocapillary concentrations) are given at the end of the table.

[1] Pohl, *Arch. f. exper. Path. u. Pharm.*, 1891, Vol. 28, p. 239.

[2] Hedin, *Pflüger's Archiv*, Vol. 68, p. 229.

[3] Warburg and Wiesel, *Pflüger's Archiv*, 1912, Vol. 144, p. 472.

[4] *Loc. cit.*, p. 471.

[5] *Cf.* Traube, "Theorie der Narkose," *loc. cit.*

TABLE IV.

Physiological Effect.	Concentrations of Alcohol (Mols per Liter) for Producing Effect.							
	Methyl.	Ethyl.	Propyl.	Butyl.	Amyl.	Hexyl.	Heptyl.	Octyl.
Narcosis of tadpoles (Overton)[1]	0.57	0.29	0.11	0.038	0.023	.....	.....	.0004
Narcosis of *Arenicola* larvæ[2]	2.2	1.1	0.34	0.09	0.03	.....	.....	.001
Prevention of cleavage in *Arbacia* eggs[3]	.....	0.87	0.27	0.086	0.037	.....	.....	.001
Checking of development in *Strongylocentrotus* eggs (Führer)[4]	0.72	0.41	0.136	0.045	.....	.....	0.0017	.0005
Narcosis of *Daphnia* (Loeb)[5]	1.2	0.6	0.2	0.12	0.04	.....	.....	.....
Production of heliotropism *Daphnia* (Loeb)[5]	0.6	0.2	0.05–0.1	.04	.....	.....	.....	.....
Haemolysis (Führer and Neubauer)[6]	7.34	3.24	1.08	0.318	0.091	0.034	0.012	.004
Decreasing oxidations by 50 per cent. in blood-corpuscles (Warburg)[7]	5.0	1.6	0.8	0.15	0.045	.....	.....	.....
Inhibition of fermentation by ether-extracted yeast (Warburg)[8]	5.0	3.5	1.3	0.54	0.23	.....	.....	.....
Depression of isolated tortoise ventricle by 50 per cent. (Vernon)[9]	1.1	0.53	0.23	0.05	0.02	.....	.....	.....
Destruction of indophenol oxidase (Vernon)[10]	10.5–14	4.8–8	1.5–2.75	0.32–0.9	.....	.....	.....	.....
Precipitation of nucleo-proteid of liver (Battelli and Stern)[11]	5.7	2.38	1.12	0.45	0.21	.....	.....	.....
Destruction of oxydon of ox-muscle (Battelli and Stern)[11]	7.54	3.57	1.16	0.44	0.19	.....	.....	.....
Decrease in action of yeast invertase (Meyerhof)[12]	3.0	1.3	0.5	0.27	0.21	.....	.....	.....
Concentrations of isocapillary solutions	14.0	5.0	1.6	0.46	0.14	.....	.....	.....

[1] Overton, "Studien über die Narkose," p. 101.

[2] R. S. Lillie, *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 372; 1913, Vol. 31, p. 255.

[3] R. S. Lillie, *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121. The concentrations of alcohols preventing the activation of unfertilized *Arbacia* eggs by pure isotonic solutions of KCNS and NaI are similar; *cf. Journ. Exper. Zoology*, 1914, Vol. 16, p. 591 (see table, p. 607).

[4] Führer, *Zeitschr. f. Biol.*, 1912, Vol. 57, p. 465.

[5] J. Loeb, *Biochem. Zeitschr.*, 1909, Vol. 23, p. 93.

[6] Führer u. Neubauer, *Arch. f. exper. Path. u. Pharm.*, 1907, Vol. 56, p. 333.

[7] Warburg, *Zeitschr. f. physiol. Chem.*, 1910, Vol. 69, p. 452.

[8] Warburg and Wiesel, *Pflüger's Archiv*, 1912, Vol. 144, p. 472.

[9] H. M. Vernon, *Journ. Physiol.*, 1911, Vol. 43, p. 325.

[10] Vernon, *Journ. Physiol.*, 1912, Vol. 45, p. 197.

[11] Battelli and Stern: *Biochem. Zeitschr.*, 1913, Vol. 52, p. 226.

[12] Meyerhof: *Pflüger's Archiv*, 1914, Vol. 157, p. 251.

These data show that the increase of surface-activity observed on passing from one member of the series to the next is very generally associated with a proportionately similar increase of physiological activity. In general each member has from two to three times the capillary activity of its immediate predecessor; and the same holds true in a general way for its physiological activity. It is also to be noted, however, that in general the same holds true for lipoid-solubility.

If physiological activity is in fact a function of capillary activity, solutions of equal surface-tension ought to exhibit equal narcotic or other physiological effects. Traube cites various observations indicating that this is frequently the case. Thus Czapek[1] has determined for a large number of organic substances the surface-tensions of the solutions which have equal effects in liberating tannin from plant cells (the leaves of *Echeveria*); this effect is analogous to hæmolysis and depends on increase in the permeability of the plasma-membrane. The surface-tensions of equally effective solutions (against air) were found to approach a fairly constant value, about two-thirds of that of pure water. Kisch[2] also found that isocapillary solutions had equal effects in preventing germination of yeast; and H. Zuckerkandl[3] obtained similar results for the inhibition of protoplasmic streaming in plant cells. The results of observations by Fühner and Neubauer and also by Traube himself on hæmolysis are similar. Thus, taking again the series of alcohols: the surface-tensions of the least concentrated solutions which free tannin from Echeveria leaves and which inhibit the germination of yeast cells are as follows: (water = 1).

TABLE V.

Alcohol.	Critical Surface-tensions of Solutions Causing A. Exomosis of Tannin from *Echeveria* Cells.	B. Inhibition of Growth of Yeast.
Methyl	0.7	0.51
Ethyl	0.67	0.48
n-propyl	0.675	ca. 0.49
i-propyl	0.69	—
n-butyl	0.69	—
i-butyl	0.665	ca. 0.495
i-amyl	0.665	0.49

[1] *Cf.* Czapek, "Über eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen," Jena, G. Fischer, 1911.

[2] Kisch, *Biochem. Zeitschr.*, 1912, Vol. 40, p. 152.

[3] H. Nothmann-Zuckerkandl, *Biochem. Zeitschr.*, 1912, Vol. 45, p. 412.

In each instance equal physiological effects are produced by solutions of approximately equal surface-tension. Czapek finds that the same rule of equal action for isocapillary solutions holds good for ketones, esters, urethanes, and other compounds. Lately, however, Vernon,[1] Höber,[2] and others have pointed out various exceptions to this rule; thus chloroform, chloral hydrate, nitromethane, and ethylene glycol begin to set free tannin in solutions of much higher surface-tensions than the above. And with the higher alcohols the surface-tensions of the effective solutions are lower than the theory requires. These deviations from the rule of isocapillarity are referred by Traube partly to chemical influences (*e. g.*, acid in chloral hydrate solutions), partly to incorrect determination of surface-tension in solutions of volatile substances like chloroform and ether, partly to the influence of viscosity. In general it appears that the more viscous compounds, *e. g.*, the higher alcohols, show equal physiological action, *e. g.*, hæmolysis, in solutions of lower surface-tension than Traube's theory demands; Traube, however, believes that this difference is due simply to slowness of penetration, incident to the high viscosity of the adsorbed layer of the narcotic at the surfaces (*e. g.*, of red corpuscles) where the essential action takes place. For solutions of approximately equal viscosity the rule of equal action with equal capillarity appears to hold true. In general isocapillary solutions of surface-active substances have less hæmolytic or other physiological action the greater their viscosity; hence equal action for isocapillary solutions is to be expected only when the viscosities are similar.[3] Even this, however, is not always the case. For instance, Loeb[4] has found that weak solutions of fatty acids, as well as weak solutions of narcotics, produce positive heliotropism in daphnids; the least effective concentrations for the first six members of the acid series were: .006*n* formic, .006*n* acetic, .005*n* propionic, .004*n* butyric, .004*n* valerianic, .002*n* caproic. The increase in effectiveness with increasing molecular weight is much more gradual

[1] Vernon, *Biochem. Zeitschr.*, 1913, Vol. 51, p. 1.

[2] Rudolf Höber, "Physikalische Chemie der Zelle und der Gewebe," 4th edition, 1914, pp. 415 *seq.*

[3] *Cf.* Traube, "Influence of Viscosity and Surface-tension in Biological Processes," *Internat. Zeitschr. f. physik-chem. Biol.*, 1914, Vol. 1, p. 275.

[4] J. Loeb, *Biochem. Zeitschr.*, 1909, Vol. 23, p. 95.

than the increase in capillary activity. The influence of the H-ion concentration enters here, and probably constitutes the preponderant factor. The part played by purely chemical action seems to be underestimated in Traube's theory. Where this factor enters, surface-activity may be of subordinate importance. Thus in the case of neutral salts solutions of equal surface-tensions may have entirely different action on colloids and hence on living cells. Traube's rule is at best an approximation; but its significance is not to be underestimated on this account. Adsorption and surface-condensation undoubtedly run parallel with capillary activity; this is a matter not only of deduction from the Gibbs-Thomson principle, but also of direct observation; and surface-forces play so large a part in biological processes that it is not surprising to find a frequent parallelism between the physiological effects of solutions and their surface-activity.

Traube in fact recognizes that the lipoid-content of cells may have an influence on the rapidity of intake of the narcotic (since lipoid-solubility will naturally favor penetration), and hence may be a factor in the narcotic action; but the narcosis itself does not depend on this solution in lipoids; "the lipoid-content influences narcotic action but does not determine it; even lipoid-free cells may be narcotized."[1] This conception, however, does not seem adequate in view of the observations of Meyer cited above, on variation of narcotic action with temperature.

It is important to note that surface-active substances affect not only biological processes but also catalyses of various kinds, especially those due to enzymes and other colloidal catalyzers (platinum, etc.), where the action is probably dependent on the character and extent of the surface between catalytic agent and medium (heterogeneous catalyses). Enzymes are colloidal catalyzers formed in cell-metabolism; as colloids it is to be expected that surface-conditions will largely influence their action. Recently Bayliss[2] has emphasized the importance of such conditions.

[1] "Theorie der Narkose," *loc. cit.*, p. 305.

[2] *Cf.* Bayliss, "The Physiological Importance of Phase-boundaries," *Science*, N. S., 1915, Vol. 42, p. 509. For the influence of anæsthetics on invertase and on colloidal platinum, *cf.* Meyerhof, *Pflüger's Archiv*, 1914, Vol. 157, pp. 251, 307; *cf.* also Warburg, *ibid.*, Vol. 155, p. 547, for their influence on the catalytic activity of finely divided carbon (animal charcoal).

He has shown that various enzymes (lipase, emulsin, urease, trypsin) retain their activity when suspended in media in which they are insoluble. This fact is best explained on the view that increased concentration of the substrate at the surface of the enzyme particles is mainly responsible for the increased reaction-velocity in its presence. This view does not explain specificity, which is probably a matter in which selective adsorption and stereochemical conditions enter as factors; but it leads to the general expectation that readily adsorbable, *i. e.*, surface-active, substances will as a class have marked influence on enzyme action.

In many organisms oxidations are the chemical processes which are most evidently influenced by narcotics; and the view that narcotic action consists essentially in a suppression of intracellular oxidations has gained wide favor, and has been supported chiefly by Verworn in Germany, and by Mathews, Loevenhart and others in America. The view that this anti-oxidative action may be exerted directly upon the oxygen-catalyzers of the cell is supported by Traube. Considerable evidence consists favorable to this view. Thus Warburg finds that inorganic iron salts accelerate oxidations in disintegrated sea-urchin eggs, and he further finds that this accelerative action is checked by urethane.[1] This interesting observation suggests the possibility that catalysis by iron plays a part in intracellular oxidations; this catalysis is checked by anæsthetics, and it is to be inferred that oxidative processes under the influence of organic catalyzers or oxidases would be similarly affected. In support of this conception Traube cites various instances where oxidative processes are checked by surface-active or narcotic substances.[2] Such instances include the decomposition of hydrogen peroxide by platinum (Bredig), the oxidation of sodium sulphite by free oxygen (Bigelow, Titoff, Young; Young finds this process checked by traces of morphine, brucine, nicotine, and especially quinine; and Traube even refers the antipyretic action of quinine to its inhibiting action on oxidation); the oxidation of phosphorus and phosphorus trioxide by free oxygen (Centnerszwer, Scharff); oxidation of stannous chloride (Young); oxidation by tissue-oxidases (Vernon, Baer

[1] Warburg, *Zeitschr. f. physiol. Chem.*, 1914, Vol. 92, p. 231.

[2] Traube, "Über Katalyse," *Pflüger's Archiv*, 1913, Vol. 153, p. 309.

and Meyerstein); catalytic oxidation of oxalic acid by animal charcoal (Warburg).[1] Thus not only oxidations under the influence of heterogeneous or colloidal catalyzers may be checked by surface-active substances, but also oxidations in homogeneous solution. This would indicate that surface-activity is not the only factor involved. On the basis of this and other facts Traube puts forward the hypothesis that narcotics are essentially negative catalyzers, especially in relation to oxidative processes.[2] The question of how this anti-oxidative effect is produced within the living cell is the essential one. Traube and others have suggested that the direct action of surface-active and narcotic substances on colloids may be a chief factor. Moore and Roaf[3] have investigated the precipitation of serum by such substances, an effect which shows a general increase with surface-activity. These authors, however, refer the effect to the formation of loose chemical combinations between the narcotic and the proteins; the quantity of chloroform and other anæsthetics dissolved by serum is several times greater than by water; they regard the excess as held by chemical union, and they attribute narcotic action to such loose protein-anæsthetic compounds which limit the chemical activities of the protoplasm, including presumably the oxidations. Warburg and Wiesel also find that narcotic substances have a precipitating action on the press-juice of yeast, and that the anti-fermentative action runs parallel with the precipitating action; and they recall the older view of Claude Bernard, according to which a semi-coagulation of the cell-colloids forms the basis of narcosis.[4] Battelli and Stern[5] find that the nucleo-proteins of cell-extracts are also precipitated by lipoid-solvent anæsthetics, and that the precipitating action runs parallel with the influence in checking the activity of cell-

[1] Warburg, *Pflüger's Archiv*, 1914, Vol. 155, p. 547.

[2] Winterstein ("Heat-paralysis and Narcosis," *Zeitschr. f. allg. Physiol.*, 1905, Vol. 5, p. 323) had earlier compared narcotics to the anticatalyzers or "Paralysatoren" of Bredig.

[3] Moore and Roaf, *Proc. Roy. Soc.*, 1904, Vol. 73, p. 382; 1906, Vol. 77*B*, p. 86.

[4] *Cf.* Warburg and Wiesel, *loc. cit.*; also Claude Bernard, "Leçons sur les anesthésiques et sur l'asphyxie," Paris, 1875, p. 154. Anæsthetics, however, do not affect the osmotic pressure of protein solutions, according to Meyerhof (see footnote 1, p. 346).

[5] *Loc. cit.*

oxidases or oxydones. Traube also cites observations by Schryver[1] in support of this general point of view; surface-active substances retard the gelation of certain colloidal solutions, *e. g.*, of sodium cholate under the influence of calcium salts, and the degree of retardation runs parallel to capillary activity and narcotic action. Physical alterations of the cell-celloids may thus lie at the basis of the anti-oxidative action which, according to this view, conditions the narcotic action. Other instances of this effect will be considered later. Traube expresses his essential view as follows: "the physical alterations of the cell-colloids—and by no means of the lipoids alone—form one of the most essential conditions for the slowing of chemical processes in cells, and hence also for narcotic and other toxicological processes. These physical alterations are a consequence of the depressant influence which narcotics exert upon surface-tension, and upon the internal pressure of the cell contents."[2] According to this conception the physical alteration of the colloids would be the primary effect of the narcotic, and decrease of oxidation secondary; this view is more consistent with the membrane-theory of narcosis, about to be described, than with the previously quoted view which refers narcosis to a direct anti-catalytic action. According to the membrane-theory, it is the plasma-membrane which is primarily affected; and the decrease of oxidations (when this occurs) is a secondary consequence of the change in the membrane. This view would make the direct action of the anæsthetic on oxidation-processes relatively unimportant. To regard anæsthesia as dependent on a direct anti-catalytic action seems insufficient, especially in view of the fact that the effective anti-catalytic concentrations are much higher than those required for narcosis. It should also be remembered that magnesium sulphate and other salts can act as anæsthetics—such salts can have no such direct anti-catalytic action—also that the electric current may show the same influence. The action of anæsthetics on oxidases will shortly be considered in more detail.

The general fact that surface-active substances alter the physical condition or state of aggregation of many colloids is, however, highly important in any general theory of anæsthesia.

[1] Schryver, *Proc. Roy. Soc.*, *B*, 1914, Vol. 87, p. 366.

[2] "Theorie der Narkose," p. 302.

Probably this effect is to be related to their influence on the electrical potential difference normally existing between the colloidal particles and the medium. Gouy[1] found that the potential-difference between mercury and sulphuric acid in the capillary electrometer is lowered by the presence of many surface-active or narcotic substances; similar observations were made by Abl[2] for cadmium amalgam cells, and by Grumbach[3] for various contact-potentials; and according to Traube the order of relative action in all of these cases is essentially that of capillary activity. Now precipitation or increased aggregation of colloids is typically associated with decrease in the electrical polarization of the colloidal particles; and capillary-active substances which produce this latter effect ought therefore to further such precipitation. A similar influence of anæsthetics on the potentials shown by organic membranes like apple-skin against salt solutions was observed by Loeb and Beutner;[4] the concentrations required for appreciable lowering of potentials were, however, much higher than those ordinarily required for anæsthesia. Notwithstanding this difficulty Traube suggests that a decrease in contact-potentials, as well as of surface-tension at the active surfaces in tissues like nerve, may be an important factor in the action of narcotics. To quote from Traube's recent paper on narcosis: "The narcotic substances, in collecting at the boundary-surfaces of cell-wall and cell-fluid, lower there the electrical contact-potentials, and in so doing directly prevent the transmission of motor and sensory impulses by means of nerve-centers. . . . This retarding or inhibiting action, exerted by substances of low solution-affinity (Haftdruck) to water, upon the oxidations and other intracellular processes conditioned by cell-colloids, and also upon the electrical phenomena at boundary-surfaces, is the cause of that condition which we designate as narcosis."[5] A somewhat similar view had previously been expressed by A. B. Macallum: "Chloroform, ether, alcohol, and chloral lower sur-

[1] Gouy, *Annales de chimie et de physique*, 1906, Sér. 8, Vol. 8, p. 291, and Vol. 9, p. 75.

[2] Abl, Dissertation; Bonn, 1907 (cited from Traube, *Pflüger's Archiv*, 1910, Vol. 132, p. 521).

[3] Grumbach, *Annales de chimie et de Physique* (8), 1911, Vol. 24, p. 463.

[4] Loeb and Beutner, *Biochem. Zeitschr.*, 1913, Vol. 51, p. 303.

[5] "Theorie der Narkose," p. 306.

face-tension in aqueous solutions, in blood plasma and lymph, and in all probability also the surface-tension of all cells, but especially of the nerve-cells. This would make them incapable of receiving or transmitting a nerve-impulse."[1]

The general view that narcosis is essentially a phenomenon of asphyxia or retarded oxidation is an old one, suggested by Claude Bernard and others, and has been revived in somewhat different form of recent years, chiefly through the influence of Verworn[2] and his pupils. Decrease in oxidations is in fact frequently observed during narcosis. Thus Alexander and Cserna[3] showed by direct analysis of blood a marked decrease in the oxygen-consumption of the brain during ether and morphine narcosis; oxidation-processes in the liver are also decreased under the influence of various narcotics.[4] But whether this decrease is simply a consequence of a paralysis of metabolic as well as of other functions, or whether it is the primary and determinative condition, is a question which has been answered differently by different investigators. Verworn has identified narcosis with asphyxia chiefly because of certain similarities between the physiological behavior of asphyxiated and narcotized tissues and cells. A summation of the effects of narcosis and of asphyxia is seen under certain conditions; thus Winterstein found that frogs asphyxiated by perfusion with oxygen-free salt solution until reflex activity was lost showed no recovery from the asphyxia if supplied with oxygen while still in a state of anæsthesia.[5] The condition of narcosis appears to render oxygen unavailable to the cells. Fröhlich[6] found the same rule to hold for nerve-trunks; normally oxygen revives irritability which has been lost in an oxygen-free medium (nitrogen atmosphere);

[1] A. B. Macallum, "Surface-tension and Vital Phenomena," Univ. of Toronto Studies, 1912, No. 8, p. 70.

[2] *Cf.* Verworn, "Die Narkose," Jena, 1913; *cf.* also Harvey Lectures, 1911–12, p. 52. Cl. Bernard ("Leçons sur les anesthésiques et sur l'asphyxie," pp. 92 *seq.*) discusses the question whether anæsthesia is a form of asphyxia, but rejects this view on the ground that anæsthetized animals show no signs of general asphyxia (p. 97).

[3] Alexander and Cserna, *Biochem. Zeitschr.*, 1913, Vol. 53, p. 100.

[4] *Cf.* Joannovics and Pick, *Pflüger's Archiv*, 1911, Vol. 140, p. 327; Baer and Meyerstein, *Arch. f. exper. Path. u. Pharm.*, 1910, Vol. 63, p. 441.

[5] Winterstein, *Zeitschr. f. allg. Physiol.*, 1902, Vol. 1, p. 19.

[6] Fröhlich, *Zeitschr. f. allg. Physiol.*, 1904, Vol. 3, p. 75.

but oxygen has no such effects on narcotized nerves; similar observations were made by Nagai[1] on ciliated epithelium. There is thus no recovery from asphyxia during narcosis, even with a good supply of oxygen. Nerves subjected to prolonged narcosis show the same physiological changes as after exposure to lack of oxygen (Fröhlich,[2] Boruttau[3]); the rate of conduction is slowed, the refractory period is prolonged, and repeated stimulation causes definite fatigue-effects; oxygen then restores the normal properties, but only in the absence of the anæsthetic. All of the phenomena of asphyxia appear during narcosis even in the presence of a good supply of oxygen, just as they do in an oxygen-free atmosphere in the absence of the narcotic (Fröhlich, Bondy,[4] Heaton).[5] Experiments by Ishikawa[6] on amœbæ gave analogous results; recovery from the inhibited or non-irritable condition, whether due to simple lack of oxygen or to the presence of a narcotic, requires the same condition, namely the presence of free oxygen. Other forms of inhibition, such as the heat-paralysis of the frog's central nervous system, are promoted both by lack of oxygen and presence of narcotics (Winterstein);[7] *i. e.*, anæsthesia acts in the same direction as lack of oxygen,—an indication that both conditions produce essentially the same physiological effect. Mansfeld[8] found that in the absence of oxygen tadpoles succumb more readily to anæsthesia than in its presence; the same is true of simple protoplasmic streaming in plant cells at temperatures of 30° and over (Zuckerkandl).[9] All of these facts seem to indicate that lack of oxygen produces essentially the same effects as anæsthesia; that the two actions are largely interchangeable, and hence capable of summation.

In general, however, it may be said, in criticism of such conclusions, that inhibition or prevention, under the influence of narcotics, of physiological processes which require oxygen, does

[1] Nagai, *Zeitschr. f. allg. Physiol.*, 1905, Vol. 5, p. 34.
[2] Fröhlich, *ibid.*, pp. 455, 468.
[3] Boruttau and Fröhlich, *Pflüger's Archiv*, 1904, Vol. 105, p. 444.
[4] Bondy, *Zeitschr. f. allg. Physiol.*, 1904, Vol. 3, p. 180.
[5] Heaton, *ibid.*, 1910, Vol. 10, p. 53.
[6] Ishikawa, *ibid.*, 1912, Vol. 13, p. 339.
[7] Winterstein, *ibid.*, 1905, Vol. 5, p. 342.
[8] Mansfeld, *Pflüger's Archiv*, 1909, Vol. 129, p. 69.
[9] H. Nothmann-Zuckerkandl, *Biochem. Zeitschr.*, 1912, Vol. 45, p. 412.

not demonstrate that narcotics act directly and primarily upon oxidation-processes. The proper inference is rather that vital processes, *including* those which require free oxygen, are inhibited during anæsthesia. But anæsthesia may also inhibit physiological processes which are independent of free oxygen, as Winterstein has shown in his experiments on the narcosis of anaërobic animals like Ascaris.[1] Similarly the growth of yeast under anaërobic conditions is checked by anæsthetics in the same manner as in the presence of oxygen;[2] and the nerve cord of Limulus, which continues to send out impulses in the absence of free oxygen, is anæsthetized by ether in a typical manner.[3] Some more general condition which determines the rate of oxidations, as well as of other metabolic processes and cell-activities, is more probably the one directly affected in anæsthesia. This latter view would regard the suppression of oxidations as secondary rather than primary,—an effect rather than a cause of narcosis. During anæsthesia those processes which are directly dependent on oxidations are arrested, together with those not so dependent.

Various attempts have been made to refer narcosis to a decrease in the external supply of oxygen, or to an inability of oxygen to enter the cells. Thus anæsthesia as well as sleep were at one time popularly attributed to a condition of cerebral anæmia—an obviously untenable view, since neither condition is confined to animals with brain and circulation. That the anæsthetic hinders the entrance of oxygen into cells has recently been suggested by Mansfeld;[4] the solubility of oxygen in the lipoids of the plasma membrane—and hence its rate of entrance into cells—was held to be diminished by the solution of lipoid-solvents in the lipoids; and E. Hamburger[5] attempted to show that narcotic substances actually decreased the solubility of oxygen in lipoids. These views however must be regarded as unfounded (see Winterstein's criticism).[6]

[1] Winterstein, *Biochem. Zeitschr.*, 1913, Vol. 51, p. 165.

[2] *Cf.* Warburg and Wiesel, *loc. cit.*, p. 480.

[3] A. P. Mathews, *cf.* the article of Tashiro and Adams, *loc. cit.*, p. 451.

[4] *Loc. cit.*

[5] E. Hamburger, *Pflüger's Archiv*, 1912, Vol. 143, p. 186.

[6] Winterstein, *Biochem. Zeitschr.*, 1913, Vol. 51, pp. 158 *seq.*

The facts which offer best support to the oxidation theory of narcosis appear to be those which demonstrate an actual decrease in oxygen-consumption by isolated cells and tissues under the influence of narcotics. Thus Warburg and his associates have shown that oxygen-consumption by living cells of various kinds (red blood-corpuscles, sea-urchin eggs, bacteria, liver-cells, yeast-cells, the central nervous system) is decreased by various anæsthetics,[1] and the different narcotic compounds show the same order of relative action as in anæsthesia. For example, the several alcohols lower the oxygen-consumption of birds' erythrocytes by 50 per cent. in solutions of the following concentrations.[2]

TABLE VI.

Alcohol.	Concentration of Solution Depressing Oxidations 50 Per Cent.		Concentration for Anæsthesia of Tadpoles (Overton).
	Per Cent. (by Weight).	Molecular.	
Methyl	16	5*m*	0.52–0.62
Ethyl	7.3	1.6*m*	0.27–0.31
Propyl	5	0.8*m*	0.11
n-butyl	1.1	0.15*m*	0.038
i-butyl	1.1	0.15*m*	0.045
Amyl	0.4	0.045*m*	0.023

It is to be noted that these concentrations are much higher than those usually required for anæsthesia, as comparison with Overton's results (third column) will show. Vernon also found that anæsthetics decreased the oxidation of the indophenol reagent by fresh tissues. An especially interesting fact is that anæsthesia causes a similar though less marked decrease in oxidation by dead cells and by tissue-extracts, as has been demonstrated by both Warburg and Vernon.[3] This suggests that the anæsthetizing influence is exerted directly upon the oxygen-catalyzers of the cell; and recently a number of investigators have devoted special study to the inhibiting action of anæsthetics on oxidases.

[1] For a summary of these researches *cf.* Warburg, *München. med. Wochenschr.*, 1911, Vol. 58, p. 289; for the case of isolated tissues *cf.* Usui, *Pflüger's Archiv*, 1912, Vol. 147, p. 100; for microörganisms, Warburg and Wiesel, *loc. cit.*

[2] Warburg, *München. med. Wochenschr., loc. cit.*; *Zeitschr. f. physiol. Chemie*, 1910, Vol. 69, p. 452.

[3] Warburg and Wiesel, *loc. cit.*; Vernon, *Journ. Physiol.*, 1912, Vol. 45, p. 197.

Vernon[1] has investigated the influence of various anæsthetics upon the activity of the indophenol oxidase of the vertebrate kidney. Enzymes which accelerate the oxidative formation of the blue dye, indophenol, from a mixture of α-naphthol and para-diamino-benzene are widely distributed in organisms; and Vernon's investigations on the distribution of this oxidase in the tissues of vertebrates indicate that a relation exists between the oxidase-content of a tissue and its general oxidative activity.[2] Various anæsthetics were found to decrease the oxidation and eventually to destroy the oxidase. The concentrations required to decrease activity by one half under otherwise constant conditions showed a close parallelism with those required to hæmolyze red corpuscles. In both cases the order of relative action was the same as for anæsthesia. The parallelism of lipoid-solubility with narcotic action appeared closer than that of surface-activity; and Vernon inclines to the belief that the narcotics exert their action chiefly by dissolving in the lipoids of the plasma-membrane and so altering the properties of this structure (possibly by interfering with the interaction of oxidase and peroxidase).[3] The oxidase-inhibiting concentrations are, however, far higher than the narcotizing, and correspond rather with the cytolytic concentrations, so that a direct connection seems doubtful. The work of Battelli and Stern[4] on the influence of anæsthetics on other tissue-oxidases (*e. g.*, a liver-oxidase which oxidizes succinic to malic acid) shows in general similar relations; in this case the inhibiting action was found to run closely parallel with surface-activity,—more so, according to Battelli and Stern, than with lipoid-solubility. It showed also a striking parallelism with a precipitating action on the nucleo-proteins of the tissue. As already stated, a similar precipitating action of anæsthetics has been investigated by Moore and Roaf, who agree with Battelli and Stern in regarding this action as an important factor in anæsthesia; the authors also attribute the action of anæsthetics not to their influence on lipoids alone, but rather to an alteration

[1] *Loc. cit.*

[2] Vernon, *Journ. Physiol.*, 1911, Vol. 42, p. 402.

[3] *Loc. cit.*, 1912.

[4] Battelli and Stern, *loc. cit.*

of the proteins of the tissue.[1] It seems clear that anæsthetics may directly inhibit oxidation-catalysis in tissues. But the objection again rises that the concentrations required for these effects greatly exceed those required for anæsthesia in living cells.

The relation of oxidases to cell-respiration is still obscure. Present opinion inclines to the belief that these bodies are essentially peroxide-forming compounds (oxygenases) which are activated by other accessory substances present in cells. These bodies (activators or co-enzymes) may be other organic compounds (peroxidases); it appears also that in some cases inorganic salts, especially iron salts, may play this rôle (*cf.* Warburg).[2] There is some evidence that the combination of hæmoglobin with oxygen is of the nature of a peroxide; both hæmoglobin and hæmatin may cause bluing of guaiac and exhibit other oxidase-like properties.[3] Compounds which form unstable peroxide-like unions with oxygen are regarded by certain investigators as forming the essentially irritable part of the cell; the temporary stabilization of such compounds by any physical or chemical influence would thus be equivalent to anæsthetization. This view has recently been supported in this country by Mathews;[4] he regards anæsthesia as resulting from the formation of chemical unions between the anæsthetic and protoplasm; these unions are due to the residual valences of the anæsthetic (*i. e.*, the reserve powers of union left over in many compounds after the ordinary valences are satisfied, as seen in the formation of double salts, hydrates, etc.); the number of residual valences is variable, but tends to be higher in compounds with well-marked anæsthetic property. Mathews finds a general though not complete parallelism between the number of such valences in a compound and its narcotic power. He proposes the following explanation of anæs-

[1] *Cf.* Moore and Roaf, *loc. cit.* But Meyerhof (*Pflüger's Archiv*, 1914, Vol. 157, p. 273) finds that anæsthetics, in concentrations of the anæsthetizing or inhibiting order, have no influence on the osmotic pressure of protein solutions. This observation appears to be incompatible with the view that anæsthetics act by altering the aggregation-state of proteins.

[2] *Zeitschr. f. physiol. Chem.*, 1914, Vol. 92, p. 231.

[3] *Cf.* Moitessier, *Compt. Rend. Soc. Biol.*, 1904, Vol. 11, p. 373; Czylharz and Fürth, *Beitr. zur chem. Physiol. u. Path.*, 1907, Vol. 10, p. 358; McClendon, *Journ. Biol. Chem.*, 1915, Vol. 21, p. 275.

[4] A. P. Mathews, *Internat. Zeitschr. f. physik-chem. Biol.*, 1914, Vol. 1, p. 433.

thesia: "The irritable substance in protoplasm is a molecular oxygen-protoplasmic union or a peroxide union, unstable and similar to oxy-hæmoglobin. By stimulation this unstable molecular union passes by molecular rearrangement into a stable form, oxidation taking place and carbon dioxide being directly or indirectly produced. The anæsthetic produces anæsthesia by occupying the oxygen-receptors of the cell, thus forming a non-irritable, dissociable, anæsthetic-protoplasm compound. The various facts of anæsthesia are explicable on this theory."

Now it is a striking fact that the concentrations of the lipoid-solvent anæsthetic required to inhibit oxidations under the influence of enzymes, inorganic catalysts, or dead cells are far higher than those required for true anæsthesia, and are closely similar in their order of magnitude to the cytolytic concentrations. As already mentioned, Vernon found that the concentrations at which the activity of the kidney-oxidase began to be decidedly lowered corresponded closely with those found by Fühner and Neubauer for hæmolysis; *i. e.*, in Vernon's words, "those which dissolve the lipoid membrane of the corpuscles." The concentrations required for anæsthesia in tadpoles are from eight to ten times lower. It thus seems doubtful that the anæsthetic effects can be referred to a direct inhibitory action on oxidases. The prompt and complete reversibility of anæsthesia is also a fact unfavorable to this view. According to Vernon's results, tissue-oxidases are rapidly destroyed by those concentrations of lipoid-solvent anæsthetics which reduce their activity to half its original value. We find, in fact, that in those cases where anæsthesia is associated with decrease of intracellular oxidations, the inhibitory effect is obtained in relatively low concentrations; while in order to induce an equal decrease of oxidations in tissue-extracts, or in disintegrated tissues or cells, the required concentrations must be much higher. In other words, destroying the *structure* of the tissue destroys its sensitiveness to the inhibiting action of low concentrations of the anæsthetic. This fact seems to indicate that the anæsthetic acts on living cells primarily by altering certain organized or structural elements, upon the condition of which the normal rate of oxidation and of other metabolic processes depends. Experiments on

tissue-extracts indicate that the normal oxidations in living cells like muscle-cells are far more rapid and complete than can be effected through the simple agency of the oxidases present (Fletcher and Hopkins, Warburg, Battelli and Stern)[1]; the rôle of oxidases in cell-respiration may therefore well be a subsidiary one; and if so, the fact that anæsthetics arrest cell-activities in concentrations which are without direct influence on the oxidases may be understood. The essential change in anæsthesia would then be a reversible alteration of certain structural elements that control oxidations as well as other cell-processes.

Such a view would regard oxidases as accessory rather than primary factors in cell-oxidations. If this is true, there should be no direct parallelism between decrease of oxidations and anæsthesia; and it should be possible in certain cases to secure anæsthesia without influencing oxidations. There are in fact numerous instances of complete and typical anæsthesia unaccompanied by any essential decrease in the rate of oxidations. The anæsthesia of anaërobic animals and yeast-cells has already been cited; these instances, however, may be considered equivocal,—since oxidations are equally essential to metabolism in these organisms even though molecular oxygen may not take part in the reactions. But many cases are known where the activity of aërobic organisms or tissues may be profoundly inhibited by anæsthetics, while the rate of oxidation is unaltered or only slightly decreased. Such lack of parallelism was observed by Rhode and Ogawa for the influence of chloral hydrate on the isolated heart.[2] The case of cell-division in developing eggs is an especially clear one; here the rate of oxidation is relatively slight compared with that of active muscle-cells. Warburg found that phenyl urethane in concentrations of $m/2{,}000$ arrests cell-division completely in sea-urchin eggs (*Strongylocentrotus*), while leaving oxygen-consumption essentially unchanged; in order materially to decrease oxidations (by 40 per cent.) several times the minimal anæsthetic concentration was needed ($m/500$).[3] Similar

[1] Fletcher and Hopkins, *Journ. Physiol.*, 1907, Vol. 35, p. 287; Warburg, *Zeitschr. f. physiol. Chem.*, 1911, Vol. 70, p. 413; *Pflüger's Archiv*, 1912, Vol. 145, p. 277; Battelli and Stern, *Biochem. Zeitschr.*, 1914, Vol. 67, p. 443.

[2] Rhode and Ogawa, *Archiv f. exper. Path. u. Pharm.*, 1912, Vol. 69, p. 200.

[3] Warburg, *Zeitschr. f. physiol. Chem.*, 1910, Vol. 66, p. 305; 1911, Vol. 70, p. 413.

observations were made by Loeb and Wasteneys[1] on the eggs of another sea-urchin (*Arbacia*). In order to arrest cleavage by cyanide (which directly inhibits oxidations) a concentration sufficient to lower the rate of oxidation to one third the normal was needed. The oxidations could be reduced to one half the normal without arresting cleavage. But in solutions of various anæsthetics (chloral, urethane, chloroform, methyl, ethyl, and propyl alcohols) of concentration sufficient to prevent cleavage entirely, the rate of oxidation was found to be only slightly decreased,—on the average by less than 10 per cent. In solutions of urethane, during the complete arrest of cleavage, the rate of oxidation was 98 per cent. of the control. When it is considered that oxidations may be decreased by much more than 10 per cent. (by means of cyanide, or by lowering the temperature a few degrees) without arresting cleavage, it seems clear that the slight decrease of oxidation observed in these experiments can stand in no causal relation to narcosis. It is an accessory and apparently an unessential effect. Very similar results were found in experiments with young fish embryos (*Fundulus*) at a stage when the musculature was well-developed, so that active contractions could be evoked by external stimulation (*e. g.*, by acidulated sea water). If unstimulated, the embryos lie quiet within the egg-envelope; the disturbing effects of variations in muscular activity are thus absent. It was found that complete chloroform-narcosis had little or no influence on the rate of oxidations; ether and butyl alcohol caused some decrease in oxidations (25 per cent. to 30 per cent. at the narcotizing concentrations); but in order to render the animals insensitive by direct inhibition of oxidation through cyanide, it was found necessary to reduce the oxidations to *one ninth* of their normal rate. In marine medusæ (*Gonionemus*) paralysis by cyanide required a decrease in oxidations from three to six times greater than that accompanying urethane narcosis.

The insensitivity of many cells and tissues to simple abstraction of oxygen or presence of cyanide is in striking contrast to their sensitivity to anæsthetics. Thus nerve-trunks only gradu-

[1] Loeb and Wasteneys, *Journ. Biol. Chem.*, 1913, Vol. 14, p. 517; *Biochem. Zeitschr.*, 1913, Vol. 56, p. 295.

ally lose irritability and conductivity in an oxygen-free atmosphere, or in cyanide solutions of considerable concentration (Dontas);[1] while the desensitizing effects of anæsthesia are rapid and complete. That the two effects are essentially different is further shown by the difference in the rate of recovery, which is much prompter in the case of anæsthesia. Apparently narcosis may decrease the oxidations in resting nerve trunks, as indicated by the output of carbon dioxide; this is seen in the experiments of Tashiro and Adams cited above, but the effect is comparatively slight and probably unconnected with the loss of irritability, since, as just seen, the nerve retains irritability in cyanide solutions for a long time. Compare also Winterstein's results, about to be described. Other similar instances are ciliary movement and protoplasmic rotation, both of which are only gradually checked by lack of oxygen or by cyanide, but instantly by narcotics. Recently Winterstein[2] has shown that the reflex irritability of the frog's isolated spinal cord may be entirely lost under anæsthesia without affecting the general oxidation-rate of the tissue; in fact during alcohol narcosis there was a slight but regular *increase* in oxygen-consumption. The narcotized cord differs from the non-narcotized in one chief respect; in the normal and incompletely narcotized cord stimulation causes increased oxygen-consumption; but during complete narcosis no such effect is seen; the oxygen-consumption during stimulation is the same as in the resting non-narcotized cord. Oxygen is, however, essential for the normal irritability of the cord; complete recovery from narcosis requires not only removal of the anæsthetic but also the presence of free oxygen. There may, however, be *partial* recovery even in the absence of oxygen. These facts illustrate how important oxygen is for the normal activity of the nerve-cell; but they also show that, given a supply of free oxygen, the consumption in the normal resting cord may be the same as in the narcotized cord. If narcosis were simply asphyxia, such a result would be inexplicable. A further fact inconsistent with the "asphyxia hypothesis" of narcosis is that after a narcosis lasting for days (9 days in one of

[1] Dontas, *Arch. f. exp. Path. u. Pharm.*, 1908, Vol. 59, p. 430.

[2] Winterstein, *Biochem. Zeitschr.*, 1914, Vol. 61, p. 81.

Winterstein's experiments with urethane) reflex irritability returns promptly on the removal of the anæsthetic. There is no evidence that nerve-cells can resist lack of oxygen for any such time. There is, however, ample evidence from other sides that during narcosis the normal resting oxidations of tissues continue uninterruptedly. Winterstein also finds that the oxidative removal of acid products of asphyxia takes place equally readily in normal and in narcotized nerve-cells. The central nervous system of the frog, which normally exhibits an alkaline reaction to litmus, becomes acid during asphyxia; if oxygen is then restored the alkaline reaction returns; but narcosis was found to have no influence on this effect; clearly therefore, narcosis does not interfere with these oxidations.[1]

Such observations should be correlated with those of Vernon, Warburg, Battelli and Stern cited above, indicating that tissue-oxidations are directly influenced only by relatively high concentrations of anæsthetics. Taken in conjunction with the other instances just cited, of anæsthesia with essentially unaltered rate of oxidation, they indicate that a direct suppression of intracellular oxidations (or asphyxia) is probably not the essential basis of anæsthesia. Apparently the latter condition does not depend on any alteration of purely chemical conditions, but on some influence exercised by the anæsthetizing agency on the structural or organized (or "living") substratum in which the chemical processes take place and by which their character and rate are controlled. The evidence of this will now be considered.

There appears to be a general relation between degree of organization and susceptibility to narcosis. Plants and lower organisms require higher concentrations of anæsthetic than higher animals (Overton); in vertebrates the cells most susceptible to narcosis are those of the higher brain-centers. Such cells are distinguished by high irritability and rapid variations in their activity, peculiarities which are undoubtedly a function of their special structure. It is true that if organization is destroyed many of the chemical processes of protoplasm (oxidations and fermentations) may still continue (autolysis), and may then be slowed by anæsthetics; but as already shown, much higher con-

[1] Winterstein, *ibid.*, 1915, Vol. 70, p. 130.

centrations are required to produce such effects than to anæsthetize the intact living cells. Such facts indicate that when anæsthetics influence oxidative and other metabolic processes within the cell, they do so not directly, but through their influence upon *some specially sensitive intermediary*, which is a part of the organized structure of the cell and itself controls the rate of the intracellular chemical processes. It is this intermediary which is directly influenced by the anæsthetic. Its part may be compared to that of a sensitive starter or relay in a complex mechanical or electrical system.

Various general considerations support this view. When an irritable element, *e. g.*, a muscle-cell, is stimulated by a mechanical impact, it is difficult to suppose that the primary effect of this impact is to hasten oxidative processes; it is true that an increase in oxidations does follow, but this effect represents a later stage in the complex sequence of interdependent processes constituting the response to stimulation (and of which the contraction is the most evident). If the muscle is previously treated for a short time with an anæsthetic, or if the magnesium or calcium-content of the medium is sufficiently increased, contraction no longer results. The entire sequence of processes normally following stimulation is prevented. It seems more probable that the *primary* event in the physiological sequence is the one directly interfered with; if this is prevented so also are the others. It further seems clear that in an irritable cell this primary or determinative change must be a *surface-change*; obviously that part of the irritable element which is directly in contact with the medium is the one first affected by the stimulus; and there is ample evidence that the direct action of many stimuli is confined to this surface-layer. Indirectly the activity of the whole cell is of course affected; but this must be by means of some influence transmitted from the surface throughout the cell-interior. The nature of this influence forms in fact the chief problem of the physiology of stimulation.

The fact that a surface-effect is sufficient to set in motion the whole complex apparatus of response in the cell-interior is a cardinal one in any theory of anæsthesia. Unmistakable evidence that this is the case is seen in the delicacy of the response

which sensitive organisms like protozoa show to the contact stimuli of food particles or prey; also in the prompt response of many living cells to the presence of substances which are known from experiments on permeability not to penetrate the plasma-membranes. In general these membranes in their normal state are impermeable to the neutral salts of the alkali and alkali earth metals, as Overton showed; yet variations in the proportions of such salts in the tissue-media profoundly influence the activity and irritability of living cells. Increase in the magnesium or calcium salts of the medium may cause typical anæsthesia in muscle and nerve cells. Warburg and Harvey[1] have shown that cell-division in sea-urchin eggs may be arrested by weak solutions of sodium hydrate without the alkali penetrating the cell. A. J. Clark has made similar observations for heart muscle cells, and Harvey for the contractile cells of medusæ and for protoplasmic rotation in plant-cells.[2] Irritability and automatic activity may thus be abolished by substances to which the cell-surface is impermeable. The general facts of electrical stimulation also indicate that alteration in the electrical condition of the cell-surface is the primary event in stimulation. The investigations of Nernst and his successors in the theory of electrical stimulation show that the electrical current stimulates by changing the relative concentrations of ions on the opposite faces of the semi-permeable membranes enclosing the irritable elements,—*i. e.*, by altering the electrical polarization of the surface-film or the plasma-membrane. A characteristic electrical variation, the action-current—which is best explained as the result of alterations in the electromotor properties of the cell surface—also accompanies all forms of stimulation, and this electro-motor variation is prevented by anæsthetics. It may be held, therefore, with a high degree of probability that the primary event in stimulation is a *surface-process*, consisting in some physico-chemical alteration of the modified protoplasmic surface-film (plasma-membrane) which delimits irritable cells.[3]

[1] Warburg, *loc. cit.*; E. N. Harvey, *Journ. Exper. Zool.*, 1911, Vol. 10, p. 507.

[2] A. J. Clark, *Journ. Physiol.*, 1913, Vol. 46, p. xx; Vol. 47, p. 66; E. N. Harvey, *loc. cit.*, and Yearbook of Carnegie Institution, No. 10, 1911, p. 128.

[3] For a general discussion of the evidence bearing on this problem *cf.* my article "The Relation of Stimulation and Conduction in Irritable Tissues to Changes in

Lately many investigators have concurred in this view that alterations in the physico-chemical properties of the plasma-membrane form the essential basis of anæsthesia. Whatever condition alters this structure so as to make it less capable of undergoing the changes of permeability and of electrical polarization which normally accompany stimulation—and apparently other forms of cell-activity—has an inhibiting or paralyzing effect on the cell. This general view has been reached as the outcome of a large number and variety of investigations. Overton in 1904 pointed out that the paralyzing action of potassium salts on voluntary muscle must be referred to their action on the membrane, since plasmolytic experiments indicate that such salts do not penetrate into the cell interior.[1] The view that the physiological action of neutral salts is due primarily to their influence on the plasma-membrane was later strongly supported by Höber[2] on the basis of experiments on the influence of neutral salts on the demarcation-current potential of muscle. This may be influenced in the direction either of increase or decrease by treatment with isotonic solutions of sodium and other salts. Salt solutions, like those of potassium salts, which decrease this potential—*i. e.*, produce local negative variation—apparently do so by altering the colloids of the plasma-membrane and so increasing its permeability; in general such increased permeability to ions involves decrease in the electrical polarization of the membrane (*i. e.*, negative variation); increased polarization means an alteration of the membrane in the reverse direction, *i. e.*, of *decreased* permeability. These changes of polarization and permeability result from the altered condition of the colloids forming the membrane. The colloidal system of the membrane acquires in the presence of certain salts a less dense consistency ("Auflockerung") associated with increased permeability; and a denser consistency ("Verdichtung") in the presence of others, *e. g.*, sodium iodide, etc. The microscopic appearances observed

the Permeability of the Limiting Membranes," in *Amer. Journ. Physiol.*, 1911, Vol. 28, p. 197. Also, for a more elementary treatment, the articles "The Rôle of Membranes in Cell-Processes," and "The General Physico-chemical Conditions of Stimulation," in the *Popular Science Monthly*, 1913 and 1914.

[1] E. Overton, *Pflüger's Archiv*, 1904, Vol. 105, p. 176; *cf.* p. 207.

[2] R. Höber, *Pflüger's Archiv*, 1905, Vol. 106, p. 599.

in nerve-fibers treated with pure solutions of sodium and potassium salts support this conception.[1] Changes in the electrical condition of the cell are thus indices of changes in the colloids forming the plasma-membrane, and especially in the lipoids. Now, since the stimulation-process is always accompanied by electrical variations, it seems probable that this process is itself dependent on changes in the colloids of the plasma-membrane, involving changes of permeability; correspondingly, artificial alterations in the condition of these colloids should modify the irritability of the cell.[2] In an important paper published in 1907, entitled "Contributions to the Physical Chemistry of Stimulation and Narcosis,"[3] Höber applies this general conception to the problem of narcosis essentially as follows: Stimulation is associated with an alteration in the condition of the colloids of the plasma-membrane, involving a general increase of permeability; narcotics are those agents which prevent this alteration in the condition of the protoplasmic colloids and hence prevent stimulation. The colloids chiefly concerned are the lipoids; narcosis depends on the collection of lipoid-soluble substances in the lecithin of the plasma-membrane to a certain critical concentration, and on a prevention, by means of these substances, of the colloid-process normally concerned in stimulation. Höber showed that various anæsthetics (ethyl and phenyl urethane, chloral hydrate, chloroform, hypnon) do in fact check the action of rubidium and potassium salts in causing negative variation in frogs' muscle. They also prevent the effect of potassium sulphate in causing structural changes ("Auflockerung") in nerve. The anæsthetic thus *antagonizes* the salt-action,—just as it is known to antagonize the stimulating action. Similar effects may be produced by alkali earth salts, especially of calcium. According therefore to Höber's hypothesis, the physico-chemical basis of these antagonisms, and hence of anæsthetic action in general, is an alteration of the colloids, especially the lipoids, of the

[1] Höber, *Zentralblatt f. Physiol.*, 1905, Vol. 19, p. 390.

[2] For a fuller account of Höber's views see his general article on "the physico-chemical processes in stimulation" in *Zeitschr. f. allg. Physiol.*, 1910, Vol. 10, p. 173. Also his textbook, "Physikalische Chemie der Zelle und der Gewebe," 4th edition, 1914.

[3] *Pflüger's Archiv*, Vol. 120, p. 492.

plasma-membrane, and a consequent change in the properties of this structure. The temporary increase of permeability, which according to Bernstein's theory of the bioelectric variations, forms an essential part of the stimulation-process, is thus rendered difficult or impossible.

Very clear and concrete indication that the stimulation of muscle is in fact associated with a temporary increase in the permeability of the plasma-membrane, and that anæsthetics act by prevènting this increase, was afforded by my own experiments on the larvæ of the marine annelid *Arenicola*, carried out at Woods Hole in 1908.[1] This larva is a free-swimming trochophore one third of a millimeter long, possessing a well-developed musculature and swimming by cilia, and is peculiar in having its body-cells permeated by a brown water-soluble pigment. This pigment normally remains within the cells, but under conditions of increased permeability, as on death or under the influence of cytolytic substances, it diffuses readily into the sea-water and imparts a yellow tinge to the latter. It serves therefore as a convenient indicator of increase of permeability. If larvæ are brought suddenly from sea water into a pure isotonic solution of a sodium salt (*e. g.*, 0.6*m* NaCl), a strong muscular contraction at once results, accompanied by a well-marked loss of pigment; at the same time the cilia cease movement and soon afterwards undergo breakdown, and other toxic effects follow. If, instead of a *pure* solution of NaCl, a solution containing a little $CaCl^2$ or $MgCl^2$ is used, both changes are simultaneously prevented; neither contraction nor loss of pigment is shown; the cilia remain active, and normal swimming movements continue for a time; the general toxic action of the pure salt solution is also prevented. Thus the calcium prevents at the same time both the stimulating and the permeability-increasing action of the sodium salt. It also greatly diminishes the injurious action of the latter, *i. e.*, exerts anti-toxic action. Pure solutions of KCl also cause strong muscular contractions accompanied by loss of pigment; and the effect is similarly checked by the addition of $MgCl_2$. In mixtures of KCl and $MgCl_2$ both effects vary with the Mg-content of the

[1] *Cf. Amer. Journ. Physiol.*, 1909, Vol. 24, p. 14. *Cf.* also *ibid.*, 1911, Vol. 28, pp. 210 *seq.*

solution in a closely parallel manner; solutions with relatively high Mg-content cause slight stimulation and slight loss of pigment, while in those of relatively high K-content both effects are well marked.

Entirely different effects from those of pure solutions of potassium and sodium salts are produced by pure solutions of magnesium salts. These exert typical anæsthetic action on *Arenicola* larvæ, as on other marine animals. When brought suddenly into pure isotonic $MgCl_2$ solution the larvæ show no contraction or loss of pigment; all muscular contraction immediately ceases, the body remains rigid and extended; the cilia are more resistant and remain active, and slow undirected swimming movements continue. On return to sea water muscular movement and other normal activities are at once restored. If larvæ are brought into isotonic $MgCl_2$ solution for a few minutes, and are then transferred into pure NaCl solution, the characteristic effects following transfer to the solution from sea water—stimulation and loss of pigment—are no longer seen; the organisms remain motionless and without apparent change. If they are then returned to sea water they show prompt revival. Apparently the treatment with $MgCl_2$ renders the plasma-membrane more resistant than normally to the permeability-increasing action of the pure NaCl solution, and at the same time the muscle-cells become refractory to stimulation. The following was the general conclusion drawn at the time from these facts: "$MgCl_2$ and similarly acting solutions appear to *decrease* the permeability of the tissues and so prevent the ionic transfer on which stimulation depends. The general action of anæsthetics consists in *decreasing the normal permeability*; stimulating agencies on the other hand have the reverse effect."[1] Later experiments with a variety of lipoid-solvent anæsthetics—alcohols, esters, ether, hydrocarbons—gave essentially similar results;[2] solutions of anæsthetics in pure NaCl solution, in every case where they prevented the stimulating action of the solution, also prevented the permeability-increasing action as indicated by loss of pigment; when they did not prevent this effect, they did not prevent stimulation. A general parallelism between prevention

[1] *Loc. cit.*, p. 44.

[2] *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 372; 1913, Vol. 31, p. 255.

of permeability-increasing action and prevention of stimulation was thus shown. Anæsthetics were also found to prevent other effects depending on increase of permeability, such as the toxic effects of pure solutions of Na and K salts on sea urchin and starfish eggs, as well as on *Arenicola* larvæ,[1] and also the activation of unfertilized sea-urchin eggs by pure salt solutions (KCNS, NaI).[2] In general the anæsthetics appear to exert a *stabilizing* influence on the plasma-membrane, rendering it more resistant than normally to influences that tend to increase its permeability. To this stabilizing action both the inhibitory or anti-stimulating (anæsthetic) and the protective (anti-toxic) actions of both salts and lipoid-solvent anæsthetics are due.

Recently a large body of evidence has accumulated from various sides indicating that anæsthetics either decrease the permeability normal to the resting cell, or render the plasma-membrane more resistant than normally to increase of permeability. Thus, according to Lepeschkin,[3] the entrance of dyes into plant cells (*Spirogyra*) is checked in the presence of low concentrations of ether and chloroform, and according to Szucs,[4] also by neutral salts. I have made similar observations on *Arenicola* larvæ.[5] These effects indicate a decrease in the general permeability of the plasma-membrane to diffusing substances; this change is apparently associated with a characteristic alteration in the density or physical consistency of the membrane, rendering it more than normally resistant to disintegrative or toxic agencies in general. Hence anæsthetics as well as neutral salts protect the cilia, pigment-cells and musculature of *Arenicola* larvæ against the injurious action of pure Na-salt solutions; the same is true of sea-urchin and starfish eggs. Similar observations are described by other authors. Arrhenius and Bubanovic[6] find that blood-corpuscles may be protected by anæsthetics against cytolysis in hypotonic solutions; and Traube

[1] *Ibid.*, 1912, Vol. 30, p. 1.

[2] *Journal of Experimental Zoology*, 1914, Vol. 16, p. 591. *Cf.* also *Journ. Biol. Chem.*, 1914, Vol. 17, p. 121.

[3] Lepeschkin, *Ber. d. deutsch. Bot. Gesellsch.*, 1911, Vol. 29, p. 349.

[4] Szucs, *Jahrbuch f. wissenschaftliche Botanik*, 1912, Vol. 52, p. 85.

[5] *Amer. Journ. Physiol.*, 1909, Vol. 24, p. 26.

[6] Publications of Nobel Institute, 1913, No. 32 (quoted from Höber's "Physik. Chem. d. Zelle," p. 466).

has made similar observations for solutions of cytolytic substances (hæmoglobin).[1] The increase in permeability caused by pure solutions of Na-salts in fish eggs is also hindered by alcohol and ether, according to McClendon;[2] and Loeb has described similar effects of alcohol on *Fundulus* eggs.[3] The most conclusive evidence of a decrease in permeability during narcosis is however derived from experiments on the electrical conductivity of narcotized cells; this appears to be decreased during narcosis (McClendon, Osterhout, Höber and Joel). McClendon in 1910 found the conductivity of sea-urchin eggs to be decreased by chloroform, but did not investigate the phenomenon in detail. More extensive experiments have been carried out on plant cells by Osterhout,[4] who has succeeded in showing clearly that in the presence of moderate concentrations of anæsthetic the cells of the marine alga *Laminaria* undergo increase in electrical resistance, indicating decreased permeability to ions; on removing the anæsthetic the original conductivity returns. If too high concentrations of anæsthetic were used the result was quite different; conductivity underwent marked increase and the effects were irreversible; the tissue had in fact undergone injurious or cytolytic alteration. Evidently the change corresponding to anæsthesia is the reversible change of *decreased* conductivity, indicating decreased permeability. Similar observations on blood-corpuscles have recently been made by Joel under Höber's direction.[5]

Taken in its entirety the foregoing evidence indicates that under the influence of a narcotizing agent the plasma-membrane undergoes an increase in its general stability or resistance to alteration; stimulation is prevented because this effect requires ready and rapid variations of permeability, and of these the stabilized membrane is no longer capable. Associated with this general stabilization is a decrease in the permeability to diffusing substances; the cell is more completely shut off from the disturb-

[1] Traube, *Biochem. Zeitschr.*, 1908, Vol. 10, p. 371. See also the experiments of G. H. A. Clowes, *Proc. Soc. Exper. Biol. and Med.*, 1913, Vol. 11, p. 8.

[2] McClendon, *Amer. Journ. Physiol.*, 1915, Vol. 38, p. 173.

[3] J. Loeb, *Biochem. Zeitschr.*, 1912, Vol. 47, p. 127; *cf.* p. 155.

[4] *Cf.* Osterhout, *Science*, N. S., 1913, Vol. 37, p. 111; also "Quantitative Researches on Permeability" in "The Plant World," 1913, Vol. 16, p. 129.

[5] Joel, *Pflüger's Archiv*, 1915, Vol. 161, p. 5.

ing effects of environmental changes. There is a possibility that the permeability to gases like carbon dioxide and oxygen is also decreased, but this remains uncertain at present.

It is important to note that changes in the resistance of plasma-membranes, probably essentially similar to those underlying anæsthesia, may occur under completely normal conditions, as I have recently found in experiments on dividing sea-urchin eggs. It had been shown earlier by Lyon,[1] Spaulding,[2] and Mathews[3] that during the normal cycle of cell-division these cells are much more susceptible to poisons (cyanide, acids, ether, and K-salts) at the time when the cleavage-furrow is forming, than in the period preceding or following cleavage; during cleavage there is also an increased output of CO_2.[4] A rhythm of susceptibility to poisons and of CO_2-production is thus associated with the rhythm of cleavage. This condition suggested the possibility that the essential underlying condition of this rhythm might be a rhythmical change in the properties of the plasma-membrane; and in a series of experiments with dilute sea water it was found that the eggs do in fact undergo cytolysis in hypotonic media far more readily at the time when the furrow is forming than during the intervals between cleavage.[5] In other words, the membrane is relatively sensitive to osmotic disruption during cleavage, and relatively resistant during the intervals between cleavage, *i. e.*, at the resting times when the cell is relatively highly resistant to the action of poisons. This normal state of relatively high stability may be compared to the condition which is imparted to the membranes of irritable cells by anæsthetics. Increased resistance to hypotonic solutions in the presence of anæsthetics has been observed by Arrhenius and Bubanovic in blood corpuscles, as already cited.

How does the anæsthetic produce this alteration in the properties of the membrane? Attempts to find parallels between the effects of anæsthetics on living cells and on colloidal suspensions of lipoids have not given entirely consistent results. Höber

[1] E. P. Lyon, *Amer. Journ. Physiol.*, 1902, Vol. 7, p. 56.
[2] E. G. Spaulding, BIOL. BULL., 1904, Vol. 6, p. 224.
[3] A. P. Mathews, BIOL. BULL., 1906, Vol. 11, p. 137.
[4] Lyon, *Amer. Journ. Physiol.*, 1904, Vol. 11, p. 52.
[5] R. S. Lillie: Amer. Journ. Physiol., 1916, Vol. 40, p. 130.

and Gordon[1] found that lecithin suspensions containing ether, chloroform, chloral, or amyl alcohol were less readily precipitated by calcium and barium salts than the control suspensions, *i. e.*, were protected against precipitation or *stabilized* by the anæsthetic; and they refer to this phenomenon as "narcotization of the plasma-membrane colloid lecithin." Koch and McLean,[2] however, find that no such effect is general with anæsthetics; they find chloral and ether indifferent, while chloroform protects lecithin against precipitation by $CaCl_2$; on the other hand, alcohol and paraldehyde further precipitation. These discrepancies are probably due to differences of concentration. Recent experiments of my own with a large number of anæsthetics have shown that with the great majority of such compounds lecithin emulsions may be protected to a greater or less degree against the precipitating action of $CaCl_2$ and HCl; *i. e.*, a concentration of electrolyte just sufficient to cause precipitation in the absence of the anæsthetic, fails to do so or does so incompletely in its presence. The concentrations required to produce this stabilization are however much higher than the anæsthetizing concentrations, and the different compounds vary greatly in effectiveness. The usual increase of action with increasing molecular weight in members of homologous series (alcohol, esters) was seen. But with certain compounds little or no protection was found, while a few (ethyl and methyl alcohols, and in part isopropyl alcohol, acetonitrile, and in part paraldehyde) definitely furthered precipitation. The compounds which distinctly prevented precipitation included higher alcohols (*n*-propyl, *n*-butyl, *i*-amyl, capryl), esters (ethyl nitrate, propionate, butyrate; methyl, ethyl, and phenyl urethanes), hydrocarbons (chloroform, carbon tetrachloride, nitromethane, benzol, toluol, xylol); ethyl ether, chloral hydrate, chloretone, chloralose, acetanilide, phenyl urea. In general, therefore, lipoid-solvent anæsthetics exhibit a stabilizing influence against precipitation by electrolytes, but this influence is not always present. It may depend upon altered electrical polarization of the colloidal particles, or possibly upon increase of viscosity; but it is doubtful if in itself it forms a

[1] Hober and Gordon, *Beitr. zur chem. Physiol. u. Pathol.*, 1904, Vol. 5, p. 432.

[2] Koch and McLean, *Journ. of Pharm. and Exper. Therapeutics*, 1910, Vol. 2, p. 249.

factor of any importance in true anæsthesia. The effective concentrations are too high and the effect is too variable.

A probably more significant physical change caused by lipoid-solvent anæsthetics is an increase in the viscosity of lecithin suspensions. Handowsky and Wagner[1] observed such an increase of viscosity in the presence of alcohol; and A. Thomas, working in my laboratory, has confirmed and extended these observations.[2] Thomas observed that in the case of ether the increase of viscosity in concentrated emulsions of lecithin might go so far as to cause true gelation. I have found that this effect is very general; it is well shown in lecithin emulsions of 10 to 12 per cent. concentration; these are highly viscous, but still fluid in consistency; on the addition of many anæsthetics this consistency changes to that of a soft more or less coherent and elastic gel, in some cases of sufficient firmness to permit the inversion of the test-tube without spilling. Well-marked gelation was observed with the following compounds: alcohols (*n*-propyl, *i*-propyl, *n*-butyl, *i*-amyl, capryl), esters (ethyl formate, acetate, propionate, butyrate, nitrate), ethyl ether, ethyl chloride, ethyl bromide, chloroform, carbon tetrachloride, benzol, toluol, xylol, chloretone, chloral hydrate, chloralose, paraldehyde. On the other hand, certain very efficient narcotics had no such effect, *e. g.*, methyl, ethyl and phenyl urethanes, ethyl alcohol, nitromethane and acetonitrile. Gelation, however, is to be regarded as simply an end-effect of increase in viscosity; the latter change is the essential, and this is perhaps the most general and significant of the purely physical changes produced by lipoid-solvent anæsthetics in colloidal suspensions of lipoids. Such a change will have in general a retarding influence on physical and chemical processes taking place in such a system; it will decrease diffusion-rates and hence reaction-velocities depending on such rates; more energy, mechanical or other, is required to produce any kind of change in a highly viscous system. A general hindrance to diffusion would express itself in a decrease of permeability and of electrical conductivity. The influence of changing viscosity on the electrical conductivity of lipoid suspensions remains

[1] Handowsky and Wagner, *Biochem. Zeitschr.*, 1911, Vol. 31, p. 32.

[2] A. Thomas, *Journ. Biol. Chem.*, 1915, Vol. 23, p. 359.

to be studied. Recently Loewe[1] has investigated the influence of a number of anæsthetics on the electrical resistance of solid artificial membranes impregnated with lipoids, and he has found that in some cases they cause decided decrease of conductivity, —a change to which he refers as "narcosis of the membrane." These results recall those of Osterhout on living plasma-membranes, but whether the anæsthetic effect in living cells is so direct as Loewe's experiments would indicate seems doubtful. Clowes's recent interesting conception of an interchangeability in the relative positions of the lipoid and aqueous phases of the protoplasmic emulsion—so that either the one or the other, according to conditions, may form the external or continuous phase —may throw a much-needed light on these phenomena. He suggests "that anæsthetics function by promoting the continuity of an external fatty or lipoid phase. The solubility of this lipoid film in adjacent aqueous phases being lowered, its permeability to water-soluble substances would be diminished. Since certain vital processes presumably depend upon intermittent intercommunication between adjacent aqueous phases, it may well be imagined that a temporary interruption in this communication would result in anæsthesia" (*loc. cit.*, p. 10). Such a change would involve both decreased permeability to water-soluble substances and greater stability of the membrane.

On the whole it appears highly probable that lipoid-solvent anæsthetics cause their effects through some purely *physical* change in the cell-system—more particularly in the plasma-membrane. Their chemical inactivity as a class indicates this. Processes like solution and adsorption, rather than chemical combination, probably determine their action in most cases. It is, however, best not to be too dogmatic on this point, since the distinctions between solution, chemical combination, and adsorption are probably not absolute. There is always the possibility that in certain cases the anæsthetic may form some chemical combination which interferes with chemical or other changes necessary to stimulation; the inactivation of hæmoglobin by carbon monoxide may serve as an illustration of how this is possible. Höber[2] has recently suggested that in true reversible

[1] S. Loewe, *Biochem. Zeitschr.*, 1913, Vol. 57, p. 161.

[2] Höber, *Deutsche medizinische Wochenschrift*, 1915, No. 10.

narcosis the direct effect of the anæsthetic is limited to a surface-action or adsorption affecting all of the colloids of the membrane; in higher than the narcotizing concentrations the solvent action of the anæsthetic upon the lipoids of the membrane assumes importance and leads to disruptive and hence irreversible effects. This view attributes the destructive action of anæsthetics in high concentrations to a process different from that underlying true anæsthesia. Meyer's experiments on the effects of temperature, already cited, indicate, however, that solution of the anæsthetic in the lipoids is an essential factor in the total physiological effect. It is conceivable that a slight solution of this kind, combined with the general increase in the viscosity of the protoplasmic surface-layer, may increase the stability of the membrane; while a more pronounced solution may lead to direct solvent or other structure-altering effects which destroy its semipermeability and so cause cytolysis.

It must not be forgotten, however, that lipoid-solvents form only one class of anæsthetics. Apparently all substances or conditions which stabilize the membrane in a reversible manner may exert anæsthetic action. Anæsthesia due to neutral salts, cold, or the electric current, may be understood from this point of view; salts or low temperature may stabilize the membrane by causing gelation or other direct changes of aggregation in the colloids; the electric current by altering its state of electrical polarization. Some years ago I expressed this general view as follows: "Anæsthetic action is due primarily to a modification of the plasma-membrane of the cells or irritable elements, of such a kind as to render these membranes more resistant towards agencies which under the usual conditions rapidly increase their permeability; cytolysis and stimulation, both of which depend on such increase of permeability, are hence checked or prevented. Decrease in the readiness with which the permeability is increased thus involves for an irritable tissue decreased irritability; this effect is produced by various salts, *e. g.*, of magnesium, and by ether and other lipoid-solvent anæsthetics in certain, not too high, concentrations. . . . It seems clear that for irritable tissues the state of the lipoids in the plasma-membrane largely determines the readiness with which changes of permeability—and

of the dependent electrical polarization—are induced by external agencies. Slight permeation of the lipoids with a lipoid-solvent apparently often facilitates such changes, and hence increases irritability; the present of more lipoid-solvent renders a change of permeability difficult, hence the protective or anæsthetic action; while concentrated solutions of lipoid-solvents disrupt the membrane and produce cytolytic or irreversible alterations in the cells; hence such substances in higher concentrations are markedly toxic."[1]

The question of just how this stabilizing influence is exerted is the critical one. An irritable element like a nerve-fiber or muscle-cell responds to a slight local electrical stimulation or mechanical impact; this response is apparently associated with a rapid and reversible increase of membrane-permeability; to this latter change the electrical variation is apparently due. It is this membrane-change, with the associated variation of electrical polarization, which appears to be the primary physiological event in stimulation; it spreads rapidly over the whole membrane, and the other consequences of stimulation (contraction, increased oxidation, etc.) follow upon this surface-change. The question thus involves the whole problem of the physiology of stimulation. This is not the place for a detailed discussion of the various questions implicated in this central problem. It is evident, however, that the whole process of stimulation depends on the local initiation of the excitation-state, and on the rapid conduction of this state from the point of stimulation so as to affect the entire element.[2] All of these processes depend on the physical and chemical condition of the membrane; hence altering this condition alters the whole stimulation-process.

According to this conception the sensitivity of the membrane to changes of electrical polarization is its most characteristic peculiarity.[3] The basis of this sensitivity remains to be deter-

[1] BIOLOGICAL BULLETIN, 1911, Vol. 22, p. 328; *cf.* p. 331.

[2] For a more special discussion of the conditions of *conduction*, as distinguished from the other features of the stimulation process, *cf.* my two recent papers in the *Amer. Journ. Physiol.*, 1914, Vol. 34, p. 414, and 1915, Vol. 37, p. 348.

[3] *Cf.* the concluding section of my paper on antagonisms between salts and anæsthetics, *Amer. Journ. Physiol.*, 1912, Vol. 29, p. 391 *seq.* "In anæsthesia it is to be assumed that the membrane is so altered that it fails to respond to a change in its electrical polarization by an increase in its permeability" (p. 393).

mined. It would appear that the peculiar properties of the membrane depend upon its being a *living* structure, the seat of a specific metabolism. That the characteristic semi-permeability depends on this latter peculiarity is seen in the fact that the death-process, however induced, is always associated with a marked increase in the general permeability and electrical conductivity of cells. In other words, the normal semi-permeable condition—involving, as it must, a certain constancy in the composition and physical state of the surface-film—is maintained only so long as the cell remains alive. This fact shows that semi-permeability, and the electrical polarization which is associated with this, are not simply static properties of the plasma-membrane, but are functions of a specific metabolic activity—including probably oxidations in most cases—which maintains constant the physico-chemical characteristics of the surface-layer of protoplasm. In the irritable element this metabolism appears to be altered in a definite manner by changes in the electrical polarization of the membrane; and along with these chemical alterations go alterations of permeability and, secondarily, of electrical polarization. These latter involve the production of local electrical circuits which traverse and hence stimulate the adjoining inactive portions of the irritable element; in this manner the state of excitation *spreads*, and the whole element is stimulated.[1] But this is the case provided only that the membrane retains its normal sensitivity to changes of electrical polarization; if it has previously been rendered resistant by an anæsthetic, no such effect follows; the element as a whole then shows itself irresponsive to stimulation.

[1] For a fuller discussion *cf.* my papers on the conditions of conduction in irritable tissues, already cited.